

**Special Publication No. 22-16**

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# **Kenai Peninsula Invasive Fish Investigations, Eradication, and Native Fish Restoration, 2017–2020**

by

**Rob Massengill**

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October 2022

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Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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Weights and measures (metric)		General		Mathematics, statistics	
centimeter	cm	Alaska Administrative Code		all standard mathematical signs, symbols and abbreviations	
deciliter	dL		AAC		
gram	g	all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.	alternate hypothesis	H <sub>A</sub>
hectare	ha			base of natural logarithm	e
kilogram	kg	all commonly accepted		catch per unit effort	CPUE
kilometer	km	professional titles	e.g., Dr., Ph.D., R.N., etc.	coefficient of variation	CV
liter	L			common test statistics	(F, t, $\chi^2$ , etc.)
meter	m	at	@	confidence interval	CI
milliliter	mL	compass directions:		correlation coefficient (multiple)	R
millimeter	mm	east	E	correlation coefficient (simple)	r
<b>Weights and measures (English)</b>		north	N	covariance	cov
cubic feet per second	ft <sup>3</sup> /s	south	S	degree (angular)	°
foot	ft	west	W	degrees of freedom	df
gallon	gal	copyright	©	expected value	E
inch	in	corporate suffixes:		greater than	>
mile	mi	Company	Co.	greater than or equal to	≥
nautical mile	nmi	Corporation	Corp.	harvest per unit effort	HPUE
ounce	oz	Incorporated	Inc.	less than	<
pound	lb	Limited	Ltd.	less than or equal to	≤
quart	qt	District of Columbia	D.C.	logarithm (natural)	ln
yard	yd	et alii (and others)	et al.	logarithm (base 10)	log
<b>Time and temperature</b>		et cetera (and so forth)	etc.	logarithm (specify base)	log <sub>2</sub> , etc.
day	d	exempli gratia (for example)	e.g.	minute (angular)	'
degrees Celsius	°C	Federal Information Code	FIC	not significant	NS
degrees Fahrenheit	°F	id est (that is)	i.e.	null hypothesis	H <sub>0</sub>
degrees kelvin	K	latitude or longitude	lat or long	percent	%
hour	h	monetary symbols		probability	P
minute	min	(U.S.)	\$, ¢	probability of a type I error (rejection of the null hypothesis when true)	α
second	s	months (tables and figures): first three letters	Jan,...,Dec	probability of a type II error (acceptance of the null hypothesis when false)	β
<b>Physics and chemistry</b>		registered trademark	®	second (angular)	"
all atomic symbols		trademark	™	standard deviation	SD
alternating current	AC	United States (adjective)	U.S.	standard error	SE
ampere	A	United States of America (noun)	USA	variance	
calorie	cal	U.S.C.	United States Code	population sample	Var var
direct current	DC	U.S. state	use two-letter abbreviations (e.g., AK, WA)		
hertz	Hz				
horsepower	hp				
hydrogen ion activity (negative log of)	pH				
parts per million	ppm				
parts per thousand	ppt, ‰				
volts	V				
watts	W				

***SPECIAL PUBLICATION NO. 22-16***

**KENAI PENINSULA INVASIVE FISH INVESTIGATIONS,  
ERADICATION, AND NATIVE FISH RESTORATION, 2017–2020**

by

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# ABSTRACT

In 2017, the Alaska Department of Fish and Game (ADF&G) conducted gillnet and eDNA sampling surveys on the Kenai Peninsula to define the distribution of invasive northern pike. These surveys discovered invasive northern pike (*Esox lucius*) and muskellunge (*Esox masquinongy*) in several waters, two of them near waters collectively known as the Tote Road Lakes (TRL) south of Soldotna, Alaska, previously known to contain northern pike. During October 2018, ADF&G applied the piscicide rotenone to the TRL to eradicate invasive fish. The success of the eradication effort was evaluated by caged sentinel fish responses, posttreatment monitoring of rotenone concentration and persistence, and gillnetting surveys. Collectively, the evaluation results suggest all invasive fish were removed. Beginning in 2019, wild juvenile rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) were collected from Kenai River tributaries and relocated to the TRL to establish a new sport fishery. Researchers, representing numerous academic universities in coordination with ADF&G, released threespine stickleback (*Gasterosteus aculeatus*) into the TRL to restore the lone fish species known to naturally occur there. The total number of wild fish released into the TRL between 2019 and 2020 was 8,740 threespine stickleback, 3,876 juvenile rainbow trout, and 15,378 juvenile coho salmon. The rainbow trout and coho salmon used for release into the TRL were wild stocks from the Kenai River drainage. Such releases of rainbow trout and coho salmon into the TRL are planned to continue through at least 2023 to provide an alternate fishery to replace the fishery for invasive fish.

Keywords: Kenai Peninsula, TRL, rotenone, northern pike, chemical treatment, restoration, invasive species, eradication, Kenai River drainage

# INTRODUCTION

Northern pike (*Esox lucius*) do not naturally occur in southcentral Alaska and are considered an invasive species despite being native to most of Alaska north and west of the Alaska Range (ADF&G 2007; Figure 1). First introduced to Alaska's southcentral region in the 1950s, northern pike are now widespread in many southcentral drainages due to natural dispersion and additional illegal introductions (ADF&G 2007).

The introduction of northern pike to the Kenai Peninsula is believed to have occurred at Derks Lake (Soldotna Creek drainage) during the early 1970s.<sup>1</sup> Since then, northern pike distribution has greatly expanded, often with human aid, and northern pike have been detected in 27 Kenai Peninsula waterbodies (Figure 2).

Invasive northern pike are associated with the decline of native fisheries throughout southcentral Alaska (Rutz 1999; Sepulveda et al. 2013; Sepulveda et al. 2015; Glick and Willette 2016), and there is strong evidence that northern pike prefer soft-finned juvenile salmonids over other available prey species in southcentral Alaska (Patankar et al. 2006; Sepulveda et al. 2013). Consumption of native juvenile salmonids and other fish species by introduced northern pike has been observed elsewhere in the northwestern United States and parts of Canada (Muhlfeld et al. 2008; Walrath et al. 2015; Zelasko et al. 2016; Harvey 2019). Shallow lake morphology, common throughout much of southcentral Alaska, offers limited deep-water refugia for prey because northern pike prefer to inhabit shallow vegetated areas (Inskip 1982; Cook and Bergersen 1988; Massengill 2014a; Massengill 2014b; Dunker et al. 2018).

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<sup>1</sup> Report titled "Northern Pike (*Esox lucius*) in the Soldotna Creek System", anonymous author, available at the Soldotna ADF&G Office.

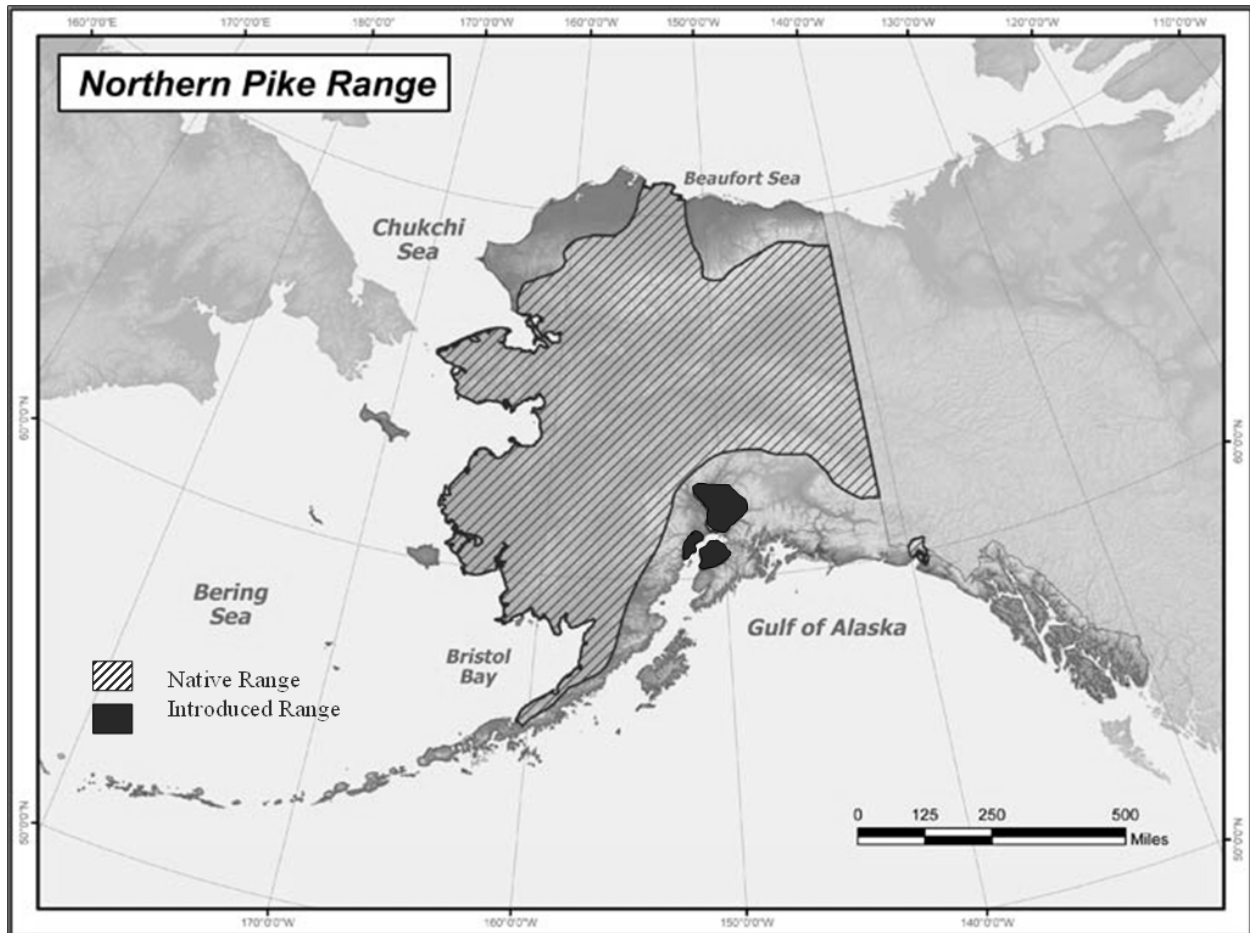


Figure 1.—Native and introduced ranges of northern pike (*Esox lucius*) in Alaska.

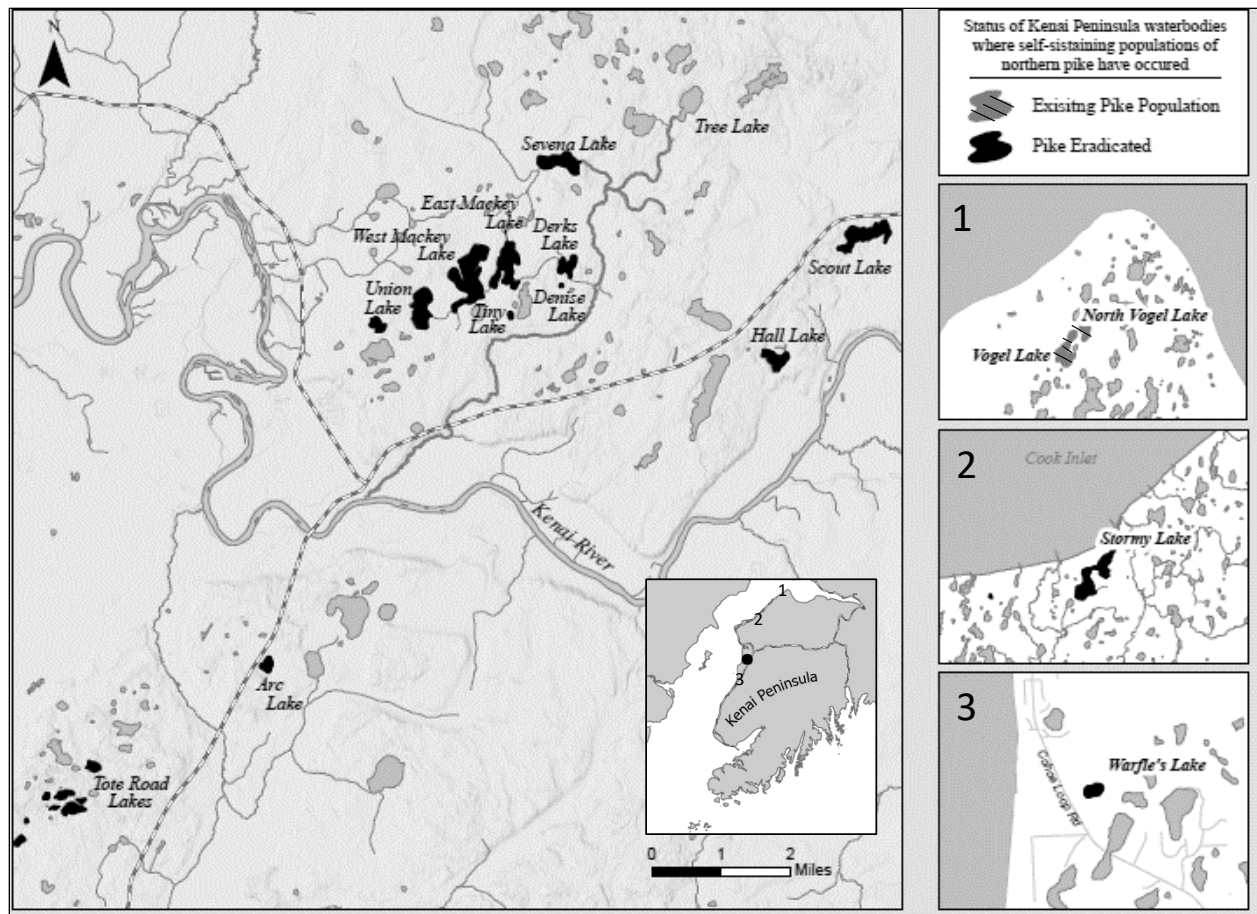


Figure 2.—Kenai Peninsula waterbodies where self-sustaining populations of northern pike have been identified, 1970s–2019.

Fish species in southcentral Alaska that extensively overlap habitat with invasive northern pike can suffer particularly poor outcomes (Dunker et al. 2018). Examples of native fish population declines in Alaska associated with northern pike invasions include the following:

- 1) Alexander Creek (Susitna River drainage) experienced severe population declines in Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kistutch*), rainbow trout (*O. mykiss*), and Arctic grayling (*Thymallus arcticus*; Rutz et al. 2020).
- 2) Threemile Creek drainage (Tyonek, West Cook Inlet), where coho salmon and sockeye salmon (*O. nerka*) populations have collapsed (Stanek et al. 2007).
- 3) Trapper and Redshirt Lakes (Susitna River drainage), where rearing sockeye salmon populations were extirpated (Glick and Willette 2016).
- 4) The western branch of the Soldotna Creek drainage (Union Lake, West Mackey Lake, East Mackey Lake, and Derks Lake) and Sevena Lake (headwaters of Soldotna Creek), where rainbow trout, coho salmon, and threespine stickleback (*Gasterosteus aculeatus*) were essentially extirpated (Massengill 2022).
- 5) Stormy Lake (Swanson River drainage), where rainbow trout, arctic char (*Salvelinus alpinus*), coho salmon, and subadult longnose suckers (*Catostomus catostomus*) were nearly extirpated (Massengill 2017b).

It is likely northern pike have impacted wild or stocked fish populations in all Kenai Peninsula waters where they have occurred; however, the paucity of historical fishery data for these waters makes quantifying these losses difficult. Fishery managers are concerned more valuable salmonid-producing habitats on the Kenai Peninsula could be invaded by northern pike. Examples of Kenai Peninsula waters deemed highly vulnerable to northern pike invasion due to their pike-preferred habitat and major salmonid producing ability include the Moose River drainage, a Kenai River tributary (Massengill 2013), and the Swanson River drainage (Palmer and Tobin 1996).

ADF&G's response to the discovery of northern pike on the Kenai Peninsula during the 1970s through 2000 was to assess their distribution and collect biological data. Investigation into the northern pike population in the Soldotna Creek drainage in the 1970s led to the planning of a removal effort using rotenone, a plant-based piscicide. Before executing the treatment, ADF&G halted the plan after evaluating that removal was unfeasible due to the scale and complexity of the drainage and the expected collateral effect on native fish populations still prevalent in the drainage.

During the mid-1980s through early 2000s, ADF&G and the U.S. Fish and Wildlife Service (USFWS) had increasing concerns about invasive northern pike on the Kenai Peninsula, in part due to reports of them occurring in new areas. Both agencies investigated the distribution of northern pike in the Kenai River drainage via surveys in the Soldotna Creek and Moose River tributaries (Palmer and Tobin 1996; McKinley 2013). Both tributaries are complex with multiple shallow lakes suitable for northern pike yet supporting significant salmonid production. No northern pike were detected in the Moose River drainage following a 1996 USFWS survey using ice fishing gear, but a 2000 ADF&G survey using gillnets and minnow traps in the Soldotna Creek drainage confirmed northern pike had invaded most of the large lakes found in the drainage and had caused the extirpation of all native fish in some (McKinley 2013). In 2001, ADF&G confirmed northern pike were present in Stormy Lake, a tributary of the Swanson River.

During 2001–2007, ADF&G mechanically suppressed select northern pike populations in the Soldotna Creek drainage and Stormy Lake using gillnets and hoop nets (Begich and McKinley 2005; Begich 2010; Massengill 2010, 2011; McKinley 2013). A block net was installed in the outlet of Stormy Lake to contain the northern pike population until they were finally eradicated with a rotenone treatment in 2012 (Massengill 2017b).

In 2008, ADF&G shifted emphasis from controlling invasive northern pike on the Kenai Peninsula using mechanical suppression methods (e.g., hoop nets, gillnets) to detecting and eradicating their populations. ADF&G's first use of rotenone to remove an invasive northern pike population occurred at Arc Lake, a small lake near Soldotna that supported a hatchery-stocked salmonid fishery (Massengill 2014b).

Between 2008 and 2017, most known northern pike populations were successfully eradicated from the Kenai Peninsula (Massengill 2014a, 2014b, 2017, 2022). This was primarily accomplished using rotenone; although at a few small lakes ( $\leq 40$  surface-acres), eradication was achieved by intensive under-ice gillnetting.<sup>2</sup> At 2 other lakes (Denise Lake and Tree Lake), northern pike disappeared by unknown cause, although a lethal low dissolved oxygen event (winterkill) is suspected for Tree Lake. By 2017, the only known northern pike population on the Kenai Peninsula was in a group of small lakes known collectively as the Tote Road Lakes (TRL) located about 5 miles south of Soldotna (Figure 2).

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<sup>2</sup> Rob Massengill, Division of Sport Fish Biologist; netting data archived in the ADF&G Soldotna Office.



Northern pike were first detected in 2 of the TRL in 1984.<sup>3</sup> Although scant fishery data exists for the TRL prior to the discovery of northern pike, it is believed threespine stickleback was the only native fish species occurring naturally. Anecdotal reports by area residents suggest that in some TRL waters, rainbow trout and Chinook salmon were periodically illegally released beginning in the 1970s, which provided some sport fishing opportunities until northern pike became prevalent. A 2006 gillnet survey of the TRL area confirmed northern pike were present in at least 6 lakes, and that no other fish species were detected in any of the lakes occupied by northern pike (Massengill 2011). This report documents ADF&G's efforts to fully assess the distribution of northern pike in the TRL area, the effort to eradicate them, and the creation of an alternative salmonid sport fishery.

## **OBJECTIVES**

The goal of this project was to restore the aquatic habitat of the TRL with the objective of eradicating invasive northern pike and muskellunge populations therein in 2018. Project tasks included the following:

- 1) Conduct fish surveys at all waters within a 1.5-mile radius of Hope Lake in 2017.
- 2) Conduct public scoping to develop a plan to eradicate the northern pike population from the TRL.
- 3) Fulfill all permitting obligations required to eradicate northern pike from the TRL.
- 4) Execute a TRL pike eradication (rotenone treatment) in the fall of 2018.
- 5) Monitor the TRL before and after treatment to include biological and water quality monitoring.
- 6) Restore native threespine stickleback to the TRL in 2019 and annually release wild salmonids (rainbow trout and [or] coho salmon) into the TRL during 2019–2023.

## **METHODS**

### **CLEARANCES FOR TREATMENT**

Many permits and approvals were required for this project, which were obtained by ADF&G. All approvals are available at the ADF&G Soldotna office and summarized below.

#### **Federal Level Approval**

- 1) An environmental assessment (EA) for the TRL Restoration was submitted to the United States Fish and Wildlife Service (USFWS) on 24 May 2018 and is archived in the Soldotna ADF&G Soldotna office. For the preferred alternative (rotenone treatment), the USFWS issued A Finding of No Significant Impact (FONSI) on 1 August 2018. The FONSI is available online at [Final FONSI Tote Road Pike Lakes\\_08-01-2018.pdf \(alaska.gov\)](https://www.alaska.gov/ADFG/FONSI_Tote_Road_Pike_Lakes_08-01-2018.pdf)

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<sup>3</sup> Dave Athons, retired Soldotna ADF&G Sport Fish biologist, 1984 memo archived in the Soldotna Office.

## State Level Approvals

- 1) An Alaska Department of Environmental Conservation (DEC) *Pesticide Use Permit* (PUP) was issued on 18 May 2018 that allowed the application of rotenone to the TRL.
- 2) An electronic Notice of Intent (eNOI) was submitted by ADF&G to the DEC *Alaska Pollution Discharge Elimination System* (APDES) program to allow the discharge of rotenone. The permit request was approved on 27 April 2018. The eNOI permit number is #AKG870004 and it required certification by the ADF&G Statewide Invasive Species Program Leader. As required by APDES for the eNOI, ADF&G completed a *Pesticide Discharge Management Plan* (PDMP). Both the eNOI approval and PDMP are archived in the ADF&G Soldotna Office.
- 3) An Alaska Department of Natural Resources *Land Use Permit* (LUP) decision was issued on 18 September 2018 stating an LUP would not be required because the requested activity (rotenone application) would not occur on general state shorelands.
- 4) Multiple ADF&G *Aquatic Resource Permits* were obtained (SF-2018-18, SF-2019-50, and SF-2020-143). These permits were needed for native fish collections to support rotenone exposure bioassays and sentinel fish uses and for relocation of salmonids to create an alternative sport fishery in the TRL. These permits were activated on 1 September 2018, 1 June 2019, and 1 June 2020, respectively.
- 5) A decision by ADF&G Habitat Division on 11 May 2020 determined that an *841 Fish Passage Permit* would not be needed for the temporary lake outlet barriers that were installed in the spring of 2020, which were designed to prevent released salmonids from leaving their release location.
- 6) A Cook Inlet Regional Incorporation (CIRI) *Land Use Permit* (2017-252) was issued on 28 July 2017, which permitted fish survey-related activities on CIRI lands neighboring the TRL.
- 7) The Alaska Board of Fisheries provided ADF&G written approval allowing the use of rotenone in the TRL, per AS 16.35.200, and this was received on 7 March 2018. The approval document is archived in the Anchorage ADF&G office.
- 8) Two Soldotna Borough Landfill Disposal Approvals (U2018-13 and L2018-12) were obtained on 8 August 2018. These were required to dispose of various pesticide contaminated waste generated by this project.

## Public Scoping and Notices

A list of the public scoping meetings, notices, and media generated for the TRL restoration project are provided below:

- 1) On 26–27 January 2017 ADF&G hand delivered notices to all TRL lakeside households about ADF&G plans to remove invasive northern pike from the TRL.
- 2) On 20 March 2017, an ADF&G biologist discussed TRL northern pike removal plans on the KSRM “Birds Eye View” radio talk show.
- 3) On 9 April 2017, an ADF&G biologist gave a presentation to the Kenai River Special Management Area (KRSMA) Habitat Group about the northern pike issue in the TRL and ADF&G’s plans to address it.

- 4) On 10 November 2017, an article was printed in the Peninsula Clarion regarding the issue of invasive northern pike in the TRL.
- 5) On 17 November 2017, an article about invasive northern pike on the Kenai Peninsula and the upcoming plans to address the TRL northern pike issue was featured in the Kenai National Wildlife Refuge's "Refuge Notebook" weekly column printed in the Peninsula Clarion.
- 6) On 22 November 2017, TRL lakeside residents were mailed a notice informing them of the upcoming public scoping meetings to discuss the TRL pike issue. Also, dozens of other stakeholders (non-lakeside property owners) were notified of these meetings by emailed notices.
- 7) On 11 December 2017 and 8 February 2018, ADF&G held public scoping meetings to vet the TRL restoration alternatives at the Kenai National Wildlife Refuge Visitor Center. The meetings were advertised with ADF&G press releases:  
<https://www.adfg.alaska.gov/sf/EONR/index.cfm?ADFG=region.NR&Year=2017&NRID=2505>;  
<https://www.adfg.alaska.gov/sf/EONR/index.cfm?ADFG=region.NR&Year=2018&NRID=2520>.
- 8) On 16 February 2018, the ADF&G Sport Fish Area Manager for the NKPMMA spoke about the Tote Road invasive pike removal plans on a local radio station (KDLL).
- 9) On 13 April 2018, all TRL lakeside residents were mailed a notice informing them of the public commenting periods for the DEC *Pesticide Use Permit* (PUP) and environmental assessment (EA) germane to removing northern pike from the TRL.
- 10) On 16 April 2018, ADF&G issued a press release informing the public of the public commenting periods for the TRL EA and PUP:  
<https://www.adfg.alaska.gov/sf/EONR/index.cfm?ADFG=region.NR&Year=2018&NRID=2542>.
- 11) On 8 August 2018, lakeside residences in the TRL were hand delivered notices to inform them of ADF&G's intention to remove northern pike from the TRL using rotenone in October 2018.
- 12) On 3 October 2018, an ADF&G news release announced the TRL rotenone treatment dates:  
<https://www.adfg.alaska.gov/sf/EONR/index.cfm?ADFG=region.NR&Year=2018&NRID=2669>.
- 13) On 4 April 2019, ADF&G mailed TRL lakeside residents a notice updating them on the status of the northern pike removal project and that the rotenone had fully degraded and explained ADF&G's plan to create a replacement salmonid fishery using wild fish releases.

## PROJECT PLANNING DATA COLLECTION

### Lake Mapping

To generate lake bathymetric maps and water volume estimates needed to plan the rotenone application to the TRL, water depth and location data were gathered. For all lakes except Leisure Pond, the data (sonar logs) were collected electronically and forwarded to a vendor for data processing. The Leisure Pond acre-feet volume was estimated differently due to its small size and

weedy, shallow composition, making electronic measurements impractical. Instead, multiple depth measurements (in feet) were collected with a weighted measuring line along multiple transects: 1 bisecting the lake north to south and 9 others equidistant from one another running east to west. The compiled depth measurements were averaged, then multiplied by the pond's surface acreage to produce a volume estimate in acre-feet. Surface acreage was calculated using a polygon measuring tool provided in Google Earth.

For the other lakes, paired depth and location data were collected using a boat-mounted Lowrance HDS 7 Touch depth finder and chart plotter with a depth-sounding transducer. The Lowrance HDS 7 Touch unit simultaneously records data at a user-selected "ping rate" (5 to 20 signals per second). Mapping guidelines suggest boat operators keep boat speed less than 20 mph or below a speed that prevents cavitation from affecting the transducer. These data were typically collected by first mapping the lake perimeter as close to shore as possible, followed by a second pass more offshore (about 7 m) from the initial pass. Subsequent mapping was done by traveling straight-line transects spaced less than 10 m apart and orientated parallel with the longest straight-line distance of the waterbody or bay being mapped. Mapped transect swaths were visually monitored on the Lowrance HDS 7 Touch screen during mapping. The Lowrance HDS 7 Touch mapping data were stored on an SD card.

Once mapping was completed, all data records were uploaded to ciBioBase, a subscription-based software service provided by Contour Innovations, LLC. ciBioBase serves as a cloud-based GIS software platform that automates data processing of the Lowrance HDS sonar logs. At ciBioBase, mapping and depth data undergo editing for erroneous data and interpolation using algorithms. Optional products include bathymetry maps, processed depth data records, volume estimates, and vegetation reports.

Processed lake depth data were used by an ADF&G analyst with GIS specialty to compute lake partition volume estimates. Creating volume estimates for lake partitions allows for a more uniform distribution of rotenone during application because each lake partition is treated individually based on its volume.

To create lake partition volume estimates, processed depth, location, and lake outline data were input into ArcGIS wherein a digital elevation model (DEM) of the lake bottom was made. ArcGIS provides a single command to create the DEM from point bathymetry data. The command is called "TOPO to Raster" and it interpolates a hydrologically correct raster surface from point, line, or polygon data. The lake outline was digitized manually from imagery layers produced by the Kenai Peninsula Borough that were already orthorectified and georeferenced. An ArcGIS tool called "Surface Volume" calculated the projected area, surface area, and volume of a surface relative to a given reference plane. A custom GIS software tool took user supplied lake partition polygons and associated lake depth grid data to compute area and volume for lake partitions (Jason Graham, ADF&G Analyst/Programmer, Anchorage, Alaska, personal communication).

## **Water Quality**

Monthly water quality data were collected from all northern pike infested lakes in the TRL for 1 year before and 1 year after their respective rotenone treatments. The water quality monitoring provides information useful for rotenone treatment planning such as understanding the anticipated water quality conditions that could affect rotenone persistence (Finlayson et al. 2010) and to document if major treatment-associated changes to water quality occur.

Water temperature, pH, dissolved oxygen, and specific conductivity (millisiemens per cm [mS/cm]) data were recorded using a Quanta Hydrolab. Water quality data were collected from near bottom to lake surface in 1-meter increments at a single site located near the deepest part of each lake. Turbidity data were measured to the nearest 0.1 meter of visibility to the naked eye using a Secchi disc at the same location where water quality data were collected.

### **Northern Pike Distribution Survey**

An ADF&G gillnet survey conducted in the TRL area during 2006 confirmed that northern pike were present in at least 6 waterbodies (Massengill 2011); all were linked in series by small seasonal outlet streams. To ensure the distribution of northern pike in the TRL area was well understood prior to planning their removal, ADF&G initiated a new survey in 2017 that utilized gillnets, minnow traps, and eDNA detection methods. The survey area included all waters within an approximate 1.5-mile radius of Hope Lake. Hope Lake is the largest of the TRL and centrally located amongst the TRL waters with northern pike populations known at the time. The goal was to locate populations of northern pike in this 1.5-mile radius.

#### ***eDNA Survey***

At most surveyed TRL area waterbodies, eDNA sampling effort was expended to achieve an estimated 90% chance of detecting a small population of northern pike ( $N = 20$ ) as described in Appendix A1. An eDNA survey was conducted before other sampling methods (gillnet and minnow trap) to reduce the likelihood northern pike DNA could be introduced to waters by contaminated sampling gear and cause false positive eDNA test results. At each waterbody surveyed, a minimum of 1 eDNA sample was collected for each 4.8 surface acres of lake or 5 samples, whichever was greater. Sampling was scheduled to occur during midsummer.

eDNA sample sites were subjectively chosen at each waterbody by the collectors who targeted weedy littoral areas that appeared to provide the best pike habitat as described by Inskip (1982). This strategy of targeting optimal habitat under a sampling rate of 1 sample/4.8 surface acres produced an average positive northern pike eDNA detection rate of 82.4% at a group of lakes with established pike populations during a 2014 Kenai Peninsula northern pike eDNA study (Massengill and Dunker 2013; Dunker et al. 2016). All eDNA sampling locations (recorded by handheld GPS), sample ID number, collection time, and collector initials were recorded manually in the field in a Rite-in-the-Rain notebook and later transcribed to a Microsoft Excel database.

Each eDNA sample consisted of a surface water grab collected in a sterilized 1-liter Nalgene bottle. To minimize eDNA contamination risk, DNA contamination prevention protocols were adopted similar to those described in Laramie et al. (2015) and Carim et al. (2016). Precautions included sterilization of all sampling equipment using a 50% bleach solution rinse (50% deionized water and 50% household bleach product containing 8.25% sodium hypochlorite), followed by deionized water rinses between all sampling sites. New latex gloves were donned for each sample collected. When possible, samples were collected via foot travel along the shoreline. Samples were either collected directly by hand or by using a swing sampler. Chest waders were sterilized with a bleach solution rinse prior to sampling at each waterbody. If a boat was needed for sampling, collectors avoided driving the boat atop or beyond a sample site until the sample was collected. A bleach rinse solution was used to sterilize the boat hull then allowed to air dry before launching at a waterbody. Immediately after collection, eDNA samples were labeled with location and date, placed in Whirl-Pak bags then placed in a cooler with ice for transport.

To test for contamination during sample collection and handling, travel, field, and lab blank samples were collected each eDNA sampling day. During specific phases of sample handling, blank samples were created by “collecting” deionized water in a sterile 1 L bottle. First, a travel blank was prepared prior to departing for the field; this involved filling a sample bottle with deionized water at the ADF&G office prior to departure and placing this bottle in the same container used to transport all samples throughout the day. Second, a field blank sample was collected using deionized water carried to the field in a sterile container and then transferred to a sample bottle while onsite at a waterbody using the same equipment used to collect the other field eDNA samples. Last, a lab blank was collected in the same room where the eDNA samples were temporarily stored and held filtered. All blank samples were lab processed identically as the field samples. Deionized water was provided by the ADF&G Limnology Lab in Soldotna.

An effort was made to filter all eDNA samples within 24 hours of collection at the ADF&G Limnology Lab in Soldotna. Prior to filtering each eDNA sample, all tweezers and filter pump assemblies were sterilized in a 50% bleach solution bath for 10–15 minutes followed by 2 deionized water baths. Before filtering a new sample, the pump and associated work area were sprayed with a 10% bleach solution and wiped dry. Last, the filter was assembled and 0.5–1.0 L of deionized water was pumped through as a final rinse. New latex gloves were worn whenever a new sample was handled.

A 120 V Geotech peristaltic pump (Geotech Environmental Equipment, Inc.; Denver, CO) was used to draw water from the sample bottle through a silicon tube filter assembly that incorporated an inline round PVC filter holder. Filters were round, 47 mm nitrocellulose mixed ester membrane (Sterlitech Corporation; Kent, WA). Filter pore size was about 1.0  $\mu\text{m}$ . The number of filters required to filter each sample varied depending on how much suspended organic material was in the sample. All filters were handled with sterilized metal tweezers. All filters used for an individual water sample were stored together in a single sterile 50 ml centrifuge tube, then sealed in a Whirl Pak bag and placed into  $-20^{\circ}\text{C}$  storage until lab processing.

All eDNA samples were processed by the USFWS Conservation Genetics Laboratory (Anchorage, AK) using qPCR assay methods described in Olsen et al. (2015). A standard curve was run for the qPCR assay to help estimate absolute quantity of DNA found in each sample.

### ***Gillnet Survey***

During TRL area gillnet surveys, an attempt was made to expend sufficient gillnetting effort to achieve an estimated 90% chance of detecting a small population of northern pike ( $N = 20$ ) as described in Appendix A2. The gillnets were manufactured by Christiansen Net Company and made of single-strand monofilament mesh with floating polypropylene hanging line and half-inch lead line. Each net was 120 ft long, 6 ft deep, with six 20 ft wide panels of variable mesh net (1 each of sequentially attached 0.50-inch, 0.63-inch, 0.75-inch, 1.00-inch, 1.50-inch, and 2.00-inch stretched mesh). The nets were fished in weedy littoral areas and generally in a hockey stick pattern with much of the net parallel to the shoreline or aquatic vegetation bed in waters 0.5 to 5.0 meters deep. Nets were tethered to shore at one end, with a buoy placed on the other end, which aided in relocating the net. Catch was identified to species, enumerated, and measured for fork length (FL) to the nearest millimeter. Fish of practical size for human consumption were donated for food or utilized for ADF&G’s educational program. To reduce the potential for gillnets to transport invasive species like elodea (*Elodea canadensis*), all gillnets were visually inspected after use, cleaned of aquatic plant fragments, then air dried and (or) disinfected with a bleach

solution soak and freshwater rinse prior to redeployment in a different waterbody. Recorded sampling data include net location (collected by handheld GPS), set and pull time, and catch (species, number, and FL). All data were recorded manually in the field using a water-resistant notebook and later transcribed to a Microsoft Excel database.

### ***Minnow Trap Survey***

Minnow trapping surveys were conducted in the same waterbodies gillnetted within a 1.5-mile radius of Hope Lake. Minnow traps were cylindrical, about 46 cm in length and constructed of galvanized wire mesh with inward sloping funnel entrances. At each waterbody, at least 5 minnow traps baited with salmon eggs were fished for a minimum of 1 hour each. All catches were identified to species, enumerated, and all salmonids measured for FL to the nearest millimeter. Fish sampling data for each trap, including set location (collected by handheld GPS), set and pull time, and catch data (species, number, and FL) were recorded manually in the field in using a water-resistant notebook and later transcribed to a Microsoft Excel database.

### **NORTHERN PIKE SALVAGE**

Prior to the rotenone application, 5 of the TRL with northern pike populations were fished with gillnets to collect northern pike for food donation and to reduce potential nuisance issues associated with a rotenone-induced fish kill (e.g., decay odors, animal scavenging). The 5 lakes were chosen based on the perceived likelihood that dead fish may cause the most nuisance issues. Salvage events used the same gillnet gear, fishing techniques, and data collection methods used for the northern pike distribution surveys. The netting effort expended on each waterbody varied relative to lake size but was kept relatively constant at each lake each day. The salvage goal, as deemed feasible using available resources and time, was to net each lake until the daily northern pike catch fell below 50% of the first day's catch.

### **BIOASSAYS**

Bioassays using juvenile salmonids were needed to determine the minimum effective dose (MED) of rotenone required for the TRL rotenone treatments. The criterion for the MED is the concentration that achieves 100% mortality after 8 hours of exposure (Finlayson et al. 2010). Finlayson et al. (2010) recommended that the target rotenone concentration be at least double the MED to account for environmental and biotic factors that can impede rotenone's effectiveness. An applicator must consider the effects of organic load, pH, turbidity, temperature, sunlight intensity, and water depth when selecting a rotenone target concentration while also ensuring the target concentration is within allowable limits (Finlayson et al. 2010). For example, if a bioassay indicates a MED of 50 parts per billion (ppb) of rotenone, the target treatment concentration should be at least 100 ppb ( $2 \times 50 \text{ ppb} = 100 \text{ ppb}$ ).

Juvenile coho salmon, rather than northern pike, were collected from Soldotna Creek for the bioassays because it is difficult to catch northern pike of appropriately small size (larger fish would probably exceed the recommended 1 g fish per liter of water for assays; Finlayson et al. 2010). Coho salmon may have a higher tolerance to rotenone than northern pike (Marking and Bills 1976), so concentrations fatal to coho salmon were expected to kill northern pike.

For each bioassay, 4–6 fish (110 mm FL) were placed in a plastic bucket filled with 20 L of lake water. Added to each bucket was a preselected amount of CFT Legumine according to directions provided in Finlayson et al. (2010). The bioassays tested the active ingredient (rotenone) across

concentrations ranging from 0 ppb (control) to 200 ppb using the amounts of rotenone premixture (rotenone product diluted with water) found in Table 1. The elapsed time was recorded when fish were observed becoming impaired (i.e., unable to remain orientated, excessive surface gulping, immobile except for gill movement, or death defined by lack of gill movement). Water temperature and dissolved oxygen data were recorded in the bioassay containers using a Quanta Hydrolab to confirm if water temperature and dissolved oxygen levels had remained sustainable.

Table 1.—Reference table amounts of rotenone piscicide (CFT Legumine) premix added to various bioassay container volumes to achieve desired concentrations.

Target concentration in ppb <sup>a</sup>	Bioassay container volume	
	10 liters (L)	20 liters (L)
	Milliliters (ml) of premix <sup>b</sup>	Milliliters (ml) of premix <sup>b</sup>
12.5	2.5	5
25	5.0	10
50	10.0	20
100	20.0	40
200	40.0	80

<sup>a</sup> Target concentration refers to amount of rotenone (not total product) in parts per million.

<sup>b</sup> Premix consists of 1 mL of liquid pesticide product or 1 mg of powdered rotenone product, containing 5% rotenone, added to 1 L of water. An adjustment must be made for how much product is required for the premix if the assayed rotenone concentration is different than 5% rotenone (see Appendix B1 for product label).

## CALCULATING PRODUCT REQUIRED

CFT Legumine, a liquid form of rotenone piscicide, was selected to treat the TRL. Liquid products are considered safer than powdered products for applicators because of the lower inhalation risk compared to powdered products. The amount of CFT Legumine needed to treat each waterbody was calculated based on bioassay results and the water volume.

Example calculations for determining the amount of CFT Legumine to apply to a lake, creek, or wetland are provided below using a hypothetical target concentration of 0.8 ppm of product (40 ppb) active ingredient rotenone.

### Lake Treatment

#### *Liquid Rotenone Formulation Example*

The number of gallons of CFT Legumine ( $G_p$ ) required to treat a hypothetical lake of 400 acre-feet with a target concentration of 0.8 ppm product (40 ppb active ingredient rotenone) was calculated from the product label in this manner:

$$G_p = 0.333 \times D_c \times V_e \quad (1)$$

where

0.333 = gallons of CFT Legumine product required to treat 1 acre-foot of water at 1.0 ppm (per product label; Appendix B1),

$D_c$  = desired target concentration (0.8 ppm) of CFT Legumine, and

$V_e$  = estimated volume (400 acre-feet) for hypothetical Lake X.



Therefore, it follows that for a desired target concentration of 0.8 ppm for 400 acre-feet,  $G_p = 0.333 \times 0.8 \times 400 = 106.6$  gallons of CFT Legumine are needed.

### **Creek and Wetland Treatment**

Backpack spray applicators applied CFT Legumine to wetland areas adjacent to lakes and their tributary streams that boat applicators were unable to access. The backpack sprayers have a 4-gallon tank capacity, which requires adding 1.3 cups of CFT Legumine to 4 gallons of water (Finlayson et al. 2010). Rhodamine red dye was sometimes added to the mixture (about 1 tablespoon/tank) to aid in visually identifying areas that were sprayed.

### **LIQUID ROTENONE BOAT APPLICATION TECHNIQUES**

At the 5 largest lakes of the TRL, CFT Legumine was applied using 1 or 2 outboard powered skiffs. On the smallest 3 lakes and ponds, an outboard powered canoe (Figure 3) or an outboard powered collapsible boat (Port-a-Bote) were used. All application boats required 2 applicators, one to operate the boat and another to operate the pesticide pumping apparatus. All application boats were equipped with gas-powered semi-closed pumping apparatuses consisting of a Honda trash/water pump with intake and discharge hoses. Premixing of lake water and CFT Legumine occurred within the pump in lake water and CFT Legumine was drawn in by separate intake hoses that are joined near the pump intake.



Figure 3.—Canoe with gas-powered spray apparatus.

On the larger skiffs using the larger pump systems, the pump's water intake line was a 2-inch inner diameter (ID) hose connected to a transom-mounted aluminum intake tube of the same diameter. A  $\frac{3}{4}$ -inch ID intake hose, that branched off the water intake hose, drew CFT Legumine from the product container. The canoe and Porta-Bote application boats used a smaller pump system that had  $\frac{3}{4}$ -inch water intake and discharge hoses and a  $\frac{3}{8}$ -inch pesticide siphon hose branching off the water intake. On all application pumps, a ball valve on the CFT Legumine intake hose provided control over the pesticide siphon rate.

One of the outboard-powered application skiffs could treat deeper subsurface waters (>5 m in depth). This boat had a discharge hose that forked to feed a pair of submersible 20-foot 1.5-inch ID well pipes (Figure 4). The well pipes were secured near the boat's transom using a pair of custom-made aluminum sleeve mounts, the sleeve mounts could swivel to allow the discharging end of the pipes to move up or down in the water. Pipe depth adjustment was controlled by a hand winch mounted at the bow of the boat. The winch cable was affixed to a spreader bar connected near the discharge end of the pipes. The winch could be cranked to raise or lower the pipes. If desired, discharge could occur from the water's surface to a maximum depth of about 5.6 m.



Figure 4.—Outboard-powered skiff with deep-water application apparatus.

An electronic chart plotter and depth finder (Garmin GPSMAP 440s FishFinder) was used by outboard boat applicators to track the boat's application swath, speed, and lake depth. A printed reference chart (Appendix C1) allowed boat applicators to adjust boat speed in relation to water depth to promote a more even distribution of rotenone when using a selected pesticide premixture discharge rate of 0.75 gallons/minute. Generally, applicators would first apply piscicide to the outermost perimeter of an area and work their way inward by making increasingly smaller concentric loops while maintaining approximately 30-foot distances between application swaths.

The second large lake application skiff had a nearly identical piscicide pumping system, but without the deep-water application capability; instead, the discharge hose was connected to a spray turret near the front of the boat. The turret nozzle could tilt vertically and rotate laterally to allow applicators to direct discharge as needed (Figure 5).

The outboard powered canoe and Porta-Bote utilized a much smaller Honda water pump system that functioned on the same principle and design as the large skiff apparatuses, except the discharge hose was connected to a 30-inch long  $\frac{3}{4}$ -inch diameter pipe intended as a handheld spray wand,

which allowed applicators to horizontally spray up to 4.5 m to treat submerged wetlands difficult to access by canoe.



Figure 5.—Outboard-powered skiff with spray turret.

## ROTENONE DEACTIVATION

As required by the product labeling, if rotenone-treated waters travel outside the treatment area, chemical deactivation with potassium permanganate is required unless dilution with untreated waters renders the rotenone concentration below 2.0 ppb (Finlayson et al. 2010). Drainage from the TRL, typically less than 1 ft<sup>3</sup>/s, flows westward in a small unnamed creek and terminates at a large bog where surface water diffuses into the ground. Rotenone applied to the TRL was allowed to naturally deactivate without the application of potassium permanganate because the TRL treatment area was essentially closed and the creek draining this lake complex was unconnected to other surface waters and absorbed into a lowland bog.

## TREATMENT SUCCESS EVALUATION

### Sentinel Fish

Caged juvenile coho salmon collected from Soldotna Creek, a Kenai River tributary, served as sentinel fish to test the effectiveness of rotenone treatments in real time. The sentinel fish were collected from Soldotna Creek and used as a surrogate for northern pike because coho salmon have a higher tolerance to rotenone than northern pike (Marking and Bills 1976), so concentrations fatal to coho salmon should effectively kill northern pike as well. Caged sentinel fish were

suspended at various depths and locations throughout each treated lake and the Freds Lake outlet stream. At least 3 fish were placed in each cage. The fish were monitored periodically during and after each lake's treatment to verify lethality.

### Rotenone Sampling

Rotenone samples were collected from each TRL waterbody immediately before and periodically after the rotenone treatments to verify if rotenone was present and to monitor its concentration and persistence (Figure 6). Rotenolone, a less toxic rotenone degradation metabolite (Schnick 1974), was also analyzed from these samples. Pretreatment rotenone sampling entailed collecting a single surface grab sample (1 m below the lake surface) from each lake destined for rotenone treatment and a single grab sample from a private groundwater well, when available. Leisure Pond has no nearby lakeside groundwater wells so no well sampling occurred there. One groundwater well was located on a narrow point of land between Ranchero Lake and Hope Lake and served as a well water source associated with both lakes.

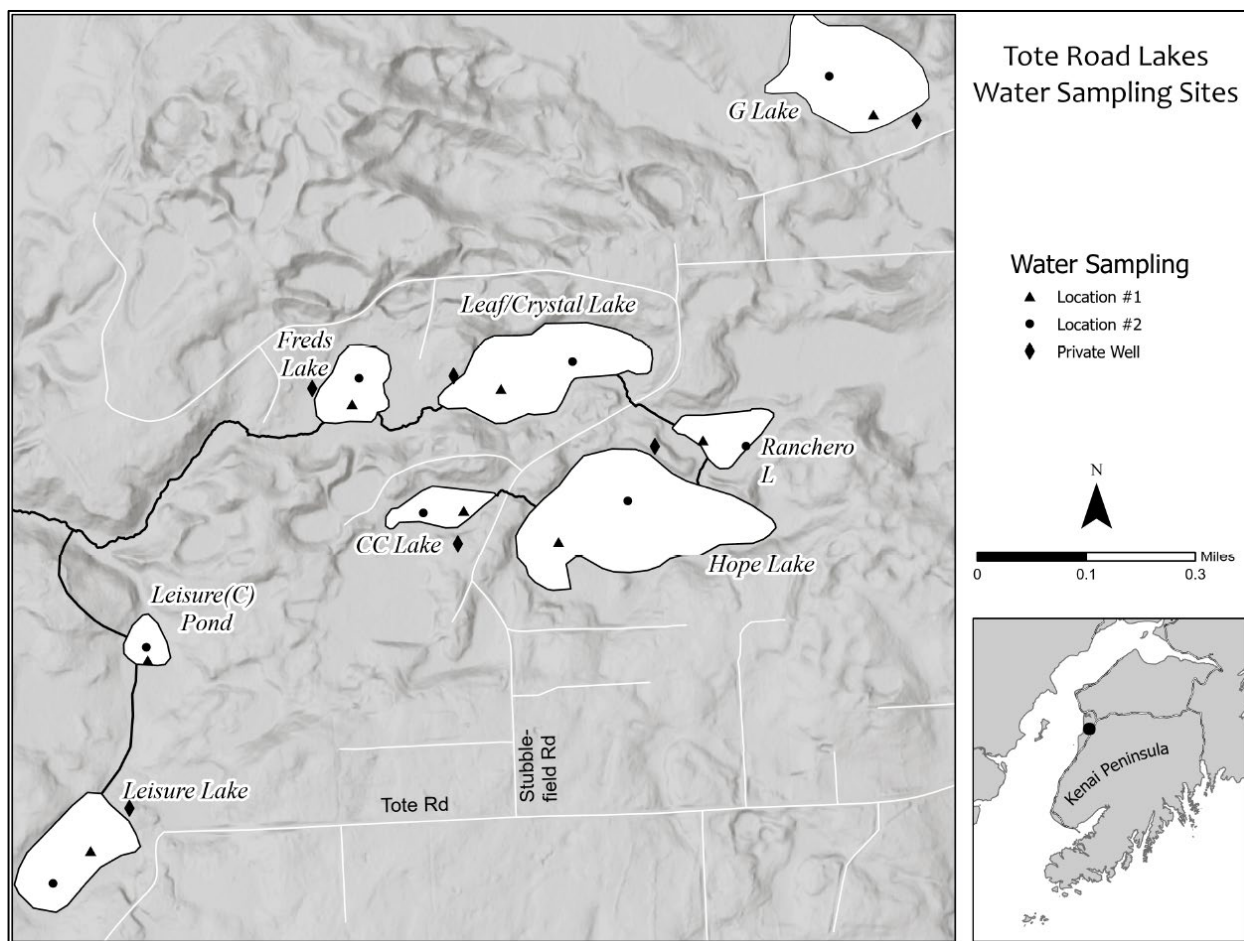


Figure 6.—Primary locations in the Tote Road Lakes (TRL) where water samples were collected from lakes and private wells to assess rotenone concentration.

Posttreatment rotenone sampling involved collecting at least 1 near-surface lake water composite sample (50:50 mixture of water from 2 locations from the same lake) and a private well-water grab sample. Additional lake rotenone samples were collected at the discretion of the project leader to

provide more insight into the distribution and persistence of rotenone. These additional samples included both surface and deep-water composite samples from select lakes. All deep-water samples were collected at least midway in the water column at locations over the deepest parts of each lake. Sample site locations were usually replicated between sampling events, but variation in sample site locations did occur when unsafe ice conditions prevented access to regular sampling sites.

In addition to sampling preselected private wells, an effort was made to accommodate other lakeside residents who requested their wells be tested for rotenone. We also collected surface water grab samples from the Freds Lake outlet stream and the Leisure Pond outlet stream opportunistically, at the discretion of the project leader. Posttreatment rotenone sampling continued periodically until the rotenone was fully deactivated ( $<2.0$  ppb; Finlayson et al. 2010).

All samples were collected in 1 L amber-colored TraceClean bottles. Lake samples were collected by lowering a 2.2 L Kemmerer sampling tube to the desired depth using a handheld line, then activating a capture mechanism (sliding a messenger weight) that triggers the closure of the tube ends. Upon retrieval of the Kemmerer tube, water was transferred to the sample bottle. Stream surface water grab samples were collected by hand by directly filling a bottle. Well water samples were collected by running water from an outside residential water spigot and letting the water run for about 10 minutes, then filling a sample bottle by hand.

All water sample bottles were labeled with location, collection date, type (well or lake; if a lake, the sample was then labeled as a deep or near-surface sample), and then placed individually in plastic bags and bubble-wrapped for protection before storing on ice inside an insulated cooler. Samples were expressed shipped to the Applied Science Engineering and Technology (ASET) laboratory at the University of Alaska, Anchorage, for processing as soon as possible, typically within 24 hours of collection. Samples were analyzed using high performance liquid chromatography (HPLC) as described by Couture et al. (2020).

### **Posttreatment Gillnet Surveys**

Gillnets were the primary method used to assess the treatment's success at removing northern pike and muskellunge from the TRL. We strove to apply enough netting effort to achieve an estimated 90% probability of detecting a northern pike population of 4 individuals at each waterbody.

Following treatment, most TRL area lakes and ponds were sampled with gillnets set during fall ice-up and fished under ice continuously, unmonitored until their removal at ice-out the following spring. The lone exception to under-ice netting was at Freds Lake because that lake froze over before we had an opportunity to deploy the nets. The posttreatment gillnet survey conducted at Freds Lake occurred shortly after ice-out in the spring of 2019. All gillnets set in the TRL area were the same as those described earlier and were located near vegetated nearshore areas typically preferred by northern pike.

## **BIOLOGICAL MONITORING BEFORE AND AFTER TREATMENT**

### **Invertebrate Surveys**

Macroinvertebrate and zooplankton surveys were conducted before and after treatment to identify taxonomic diversity present in the TRL. A representative lake (Hope Lake) was selected for surveying. A minimum of 1 pretreatment and posttreatment sampling survey was planned. Pretreatment and posttreatment surveys were conducted using the same equipment and site

locations and conducted during late August of 2018 (pretreatment) and 2019 (posttreatment). All sampling locations were recorded with a handheld GPS to ensure repeat site selection (Figure 7). At each sampling site, all captured invertebrates were combined into a single glass jar filled with denatured ethanol and labeled with the date, site location, and gear type.

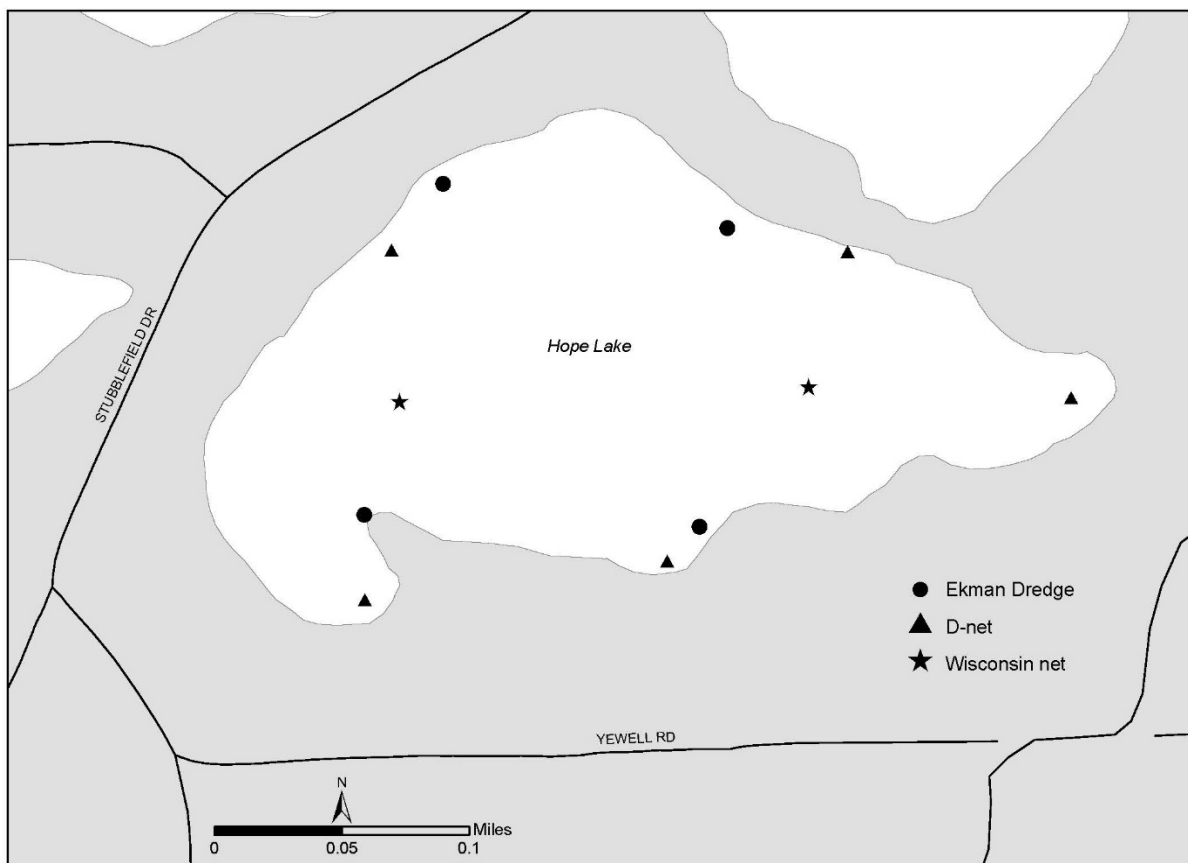


Figure 7.—Hope Lake invertebrate sampling sites by gear type

During each lake sampling survey, zooplankton collections were made with replicate vertical tows (from bottom of the lake to surface) at 2 different sites in locations near maximum lake depth using a 0.5 m diameter Wisconsin net with 153  $\mu$ m mesh. The net was lowered to near the lake bottom with a hand line and then retrieved at a rate of 1 m every 2 seconds. As the net was retrieved, captured zooplankton concentrated in the net bottom inside a screened PVC collection bucket. At the surface, the bucket was detached, and captured zooplankton were transferred to a collection jar. Zooplankton samples were generally resolved to the order or family level using illustrations found in Bachmann (1973) and taxonomic keys found in Pennak (1989).

During each survey, benthic macroinvertebrates were collected using a 9-inch Ekman Bottom Grab Sampler to collect bottom organisms from 5 offshore sites. The Ekman sampler was deployed from an anchored outboard skiff in 1.5 to 3 m of water. Collected sediment was screened to filter out invertebrates, which were removed from the screen with tweezers.

Handheld D-nets were used to sample lake invertebrates along vegetated nearshore areas (<0.6 m in depth) in 5 locations. The D-net was swept back and forth through submerged vegetation for 30 seconds. Visual observations of freshwater mussels and snails were done opportunistically in

nearshore areas. Stream sampling was done only with D-nets and conducted similarly as the lake D-net sampling. All collected macroinvertebrates were identified to the order, suborder, or family level as practical, using keys by Pennak (1989), Voshell (2002), and Merrit and Cummins (1984).

In addition to the invertebrate surveys conducted by ADF&G, researchers from the University of Quebec (Montreal), who have initiated various studies associated with the reintroduction of threespine stickleback to the TRL, conducted separate pretreatment and posttreatment invertebrate surveys. These surveys were conducted in early June of 2018 (pretreatment) and 2019 (posttreatment), and data from those surveys were shared with ADF&G.

Invertebrate surveys were conducted by sampling the littoral macroinvertebrate communities with a D-frame kick net at multiple sites in all waters of the TRL. In 2018, there were 4 sampling sites at each lake, and in 2019, there were 8 sampling sites at each lake. Sampling was performed by the “kick and sweep” method with a 500 µm D-net as recommended by the Ontario Benthos Biomonitoring Network (Jones et al. 2007) on the surface in approximately 2 m<sup>2</sup>. Samples were concentrated with a 500 µm sieve, preserved in 95% ethanol, and delivered to a laboratory at the University of Quebec in Montreal (UQAM) for identification. Identification was done to the family level following taxonomic keys (Merrit and Cummins 1984; Moisan 2010). Data from these surveys were shared with ADF&G.

### **Minnow Trapping**

Pretreatment minnow trapping was conducted in the TRL treatment area to detect if native fish species (i.e., threespine stickleback) were present prior to treatment. Researchers from UQAM conducted the pretreatment minnow trapping as previously described (*Minnow Trap Surveys* under *Northern Pike Distribution Survey*). Pretreatment minnow trapping effort varied greatly among lakes but averaged 16 traps and 49 hours of total effort per lake. The pretreatment minnow trapping surveys occurred between 2 June 2018 and 5 June 2018.

Posttreatment minnow trapping was not conducted until the summer of 2021 because results from the pretreatment surveys indicated no native fish species were present in the TRL except for threespine stickleback in G Lake. Minnow trapping surveys were conducted in all the TRL in 2021 to assess native fish populations introduced to the area following treatment; fish introduction methods are detailed below.

## **NATIVE FISH INTRODUCTION AND ASSESSMENT**

Following rotenone treatment, an important task was to restore the only native fish historically found in the TRL (threespine stickleback) and to create a new sport fishery for wild rainbow trout and landlocked coho salmon. The reintroduction of wild threespine stickleback was led by McGill University researchers and was permitted by ADF&G. The restoration plan developed by the researchers called for releasing specific forms of threespine stickleback (i.e., anadromous, freshwater, limnetic, and benthic) collected from various southcentral Alaska populations. The research objective is to conduct a long-term study of the adaptive evolutionary response of these reintroduced threespine stickleback and to document their effects on lake ecology. The researchers collected the threespine stickleback using minnow traps from various southcentral Alaska locations and transported them by highway vehicle in aerated livewell tanks to the TRL for release. Aquatic Resource Permits were issued by ADF&G for this activity and the stocking densities were determined in collaboration with ADF&G. Dr. Andrew Hendry (McGill University, Quebec, Canada) provided oversight of the stickleback releases.



Beginning with the open water season of 2019, ADF&G began a 5-year effort to collect wild juvenile rainbow trout and juvenile coho salmon to release them into TRL waters. Fish collections were done using minnow traps baited with salmon roe, and fish were collected from Soldotna Creek, Slikok Creek, and Beaver Creek, which are all Kenai River tributaries. Fish were held temporarily on site in live boxes near their point of capture, identified to species, and counted, then transported in an aerated livewell tank by highway vehicle to their release sites in the TRL. The release goal was at least 50–100 fish (in combination) per surface-acre per year, which follows guidelines advised for these species at lakes where angling effort is expected to be low (Havens et al. 1995). The combined surface-acreage of all the TRL waters is 92 acres; therefore, the total annual salmonid release target, at a minimum, ranged from 4,600 to 9,200 fish.

Assessment of native fish populations following introduction was done by gillnet and minnow trap surveys conducted between July and September of 2021 at all TRL waters. Catch data provided information about species presence, fish lengths, and catch per unit effort (CPUE). At all 8 lakes in the TRL system, 5 minnow traps were fished for a minimum of 1 hour each. All fish caught in minnow traps were identified to species and counted. Gillnet sampling effort varied at the discretion of the project leader to minimize handling mortality of native fish. The gillnet specifications and fishing methods followed those described earlier (see *Gillnet Survey* under *Northern Pike Distribution Survey*). Most fish caught in gillnets were measured for fork length (FL) to the nearest millimeter unless it would cause the fish undue stress.

Gillnet CPUE was calculated by lake and species as follows:

$$CPUE = \frac{c}{e} \quad (2)$$

where

$c$  = number of individuals (by species) captured from all nets fished in a lake, and

$e$  = total units of net fishing time per lake (1 unit = 1 hour).

## RESULTS

### WATER BODY PHYSICAL AND CHEMICAL CHARACTERIZATION

#### Lake Mapping and Partitioning

Five of the 8 infested lakes in the TRL were mapped in 2013 and the remaining 3 lakes (CC Lake, Leisure Pond, and G Lake) were mapped in 2017. Bathymetric maps were produced for all lakes treated with rotenone except for Leisure Pond, for which the volume was calculated by multiplying surface area by average depth. Appendices D1–D7 show the lake section boundaries and the amount of CFT Legumine applied to each section, including amounts applied to the lake surface or deep in the water column (see also Table 2). Orange buoys tethered to weights were placed along lake section boundaries to aid boat navigation by applicators during the treatment.



Table 2.—Summary of the amount of CFT Legumine applied to the Tote Road Lakes, 8–11 October 2018.

Waterbody name	Application date (2018)	Lake section	Acre-feet <sup>a</sup>	Percent of lake volume (%)	Gallons CFT Leg. applied to surface waters	Gallons CFT Leg. applied to subsurface waters (about 15 ft deep)	Gallons of CFT Legumine applied to all water depths
CC Lake	9 Oct	1	12.8	48	3.40	0.00	3.40
		2	13.9	52	3.70	0.00	3.70
		Total	26.6	100	7.10	0.00	7.10
Crystal Lake	10 Oct	1	48.7	18	8.64	4.33	12.97
		2	111.7	40	19.83	9.94	29.77
		3	71.3	26	12.66	6.35	19.01
		4	44.5	16	11.90	0.03	11.93
		Total	276.2	100	53.03	20.66	73.69
Freds Lake	10 Oct	1	7.0	46	1.85	0.00	1.85
		2	8.2	54	2.15	0.00	2.15
		Total	15.2	100	4.00	0.00	4.00
G Lake	11 Oct	1	67.7	24	12.00	6.02	18.02
		2	144.6	51	25.64	12.86	38.50
		3	70.2	25	12.44	6.24	18.68
		Total	282.4	100	50.08	25.12	75.20
Hope Lake	9 Oct	1	145.0	35	25.73	12.90	38.63
		2	204.9	50	36.37	18.24	54.60
		3	61.8	15	16.46	0.00	16.46
		Total	411.7	100	78.56	0.50	109.70
Leisure Lake	8 Oct	1	24.7	20	6.57	0.00	6.57
		2	53.5	43	14.23	0.00	14.23
		3	45.0	37	11.99	0.00	11.99
		Total	123.2	100	32.80	0.00	32.80
Leisure Pond	8 Oct	1	11.0	1	4.00	0.00	4.00
Ranchero Lake	9 Oct	1	12.5	30	3.34	0.00	3.34
		2	29.1	70	7.76	0.00	7.76
		Total	41.6	100	11.10	0.00	11.10
Wetlands and streams <sup>a</sup>	9–10 Oct	NA	NA	NA	1.13	0.00	1.13
		Grand total	1,187.9		241.80	46.27	318.72

<sup>a</sup> Backpack applicators applied rotenone to lake-adjacent wetlands and streams; all other waters were treated by boat applicators.

## Water Quality

Pretreatment monthly water quality sampling occurred December 2016 through November 2017 at CC Lake, Crystal Lake, Freds Lake, Hope Lake, Leisure Lake, and Ranchero Lake. At G Lake, pretreatment sampling occurred October 2017 through September 2018. At Leisure Pond, no pretreatment sampling was done due to its very small size. Posttreatment monthly water quality sampling occurred October 2018 through September 2019 at all lakes except Ranchero Lake where posttreatment sampling concluded in August 2019. In a few instances, dangerous ice conditions or equipment failure prevented the collection of monthly water quality data.

For each lake, the average monthly water temperature, specific conductance, dissolved oxygen, pH, and turbidity were graphed (Figures 8–15). In general, lake water quality parameters displayed typical interannual variation and remained similar between pretreatment and posttreatment periods, with no major drops in oxygen or turbidity that might be associated with fish kill-related nutrient loading, etc.

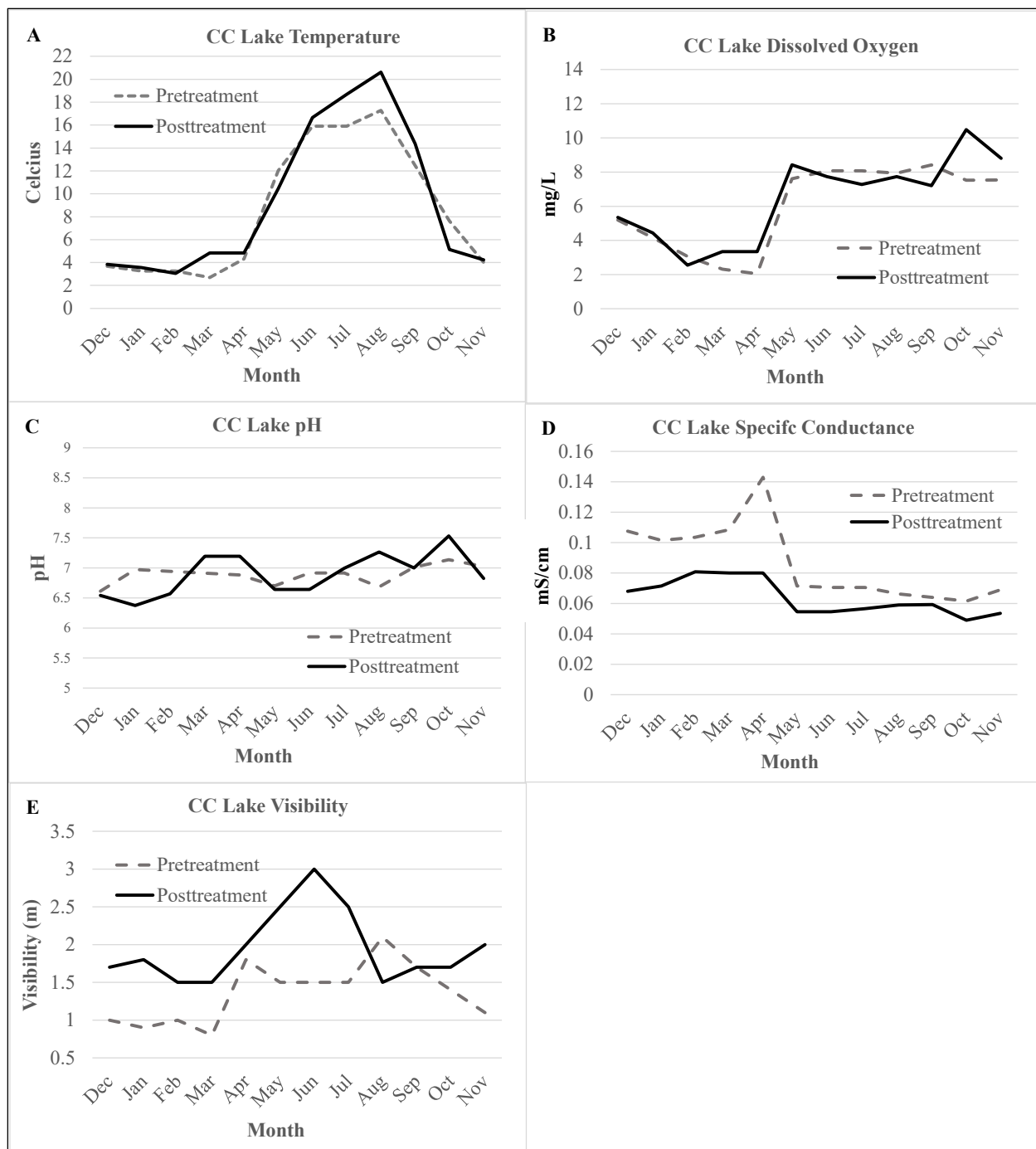


Figure 8.—CC Lake pretreatment (December 2016 through November 2017) and posttreatment (October 2018 through September 2019) average monthly water quality data.

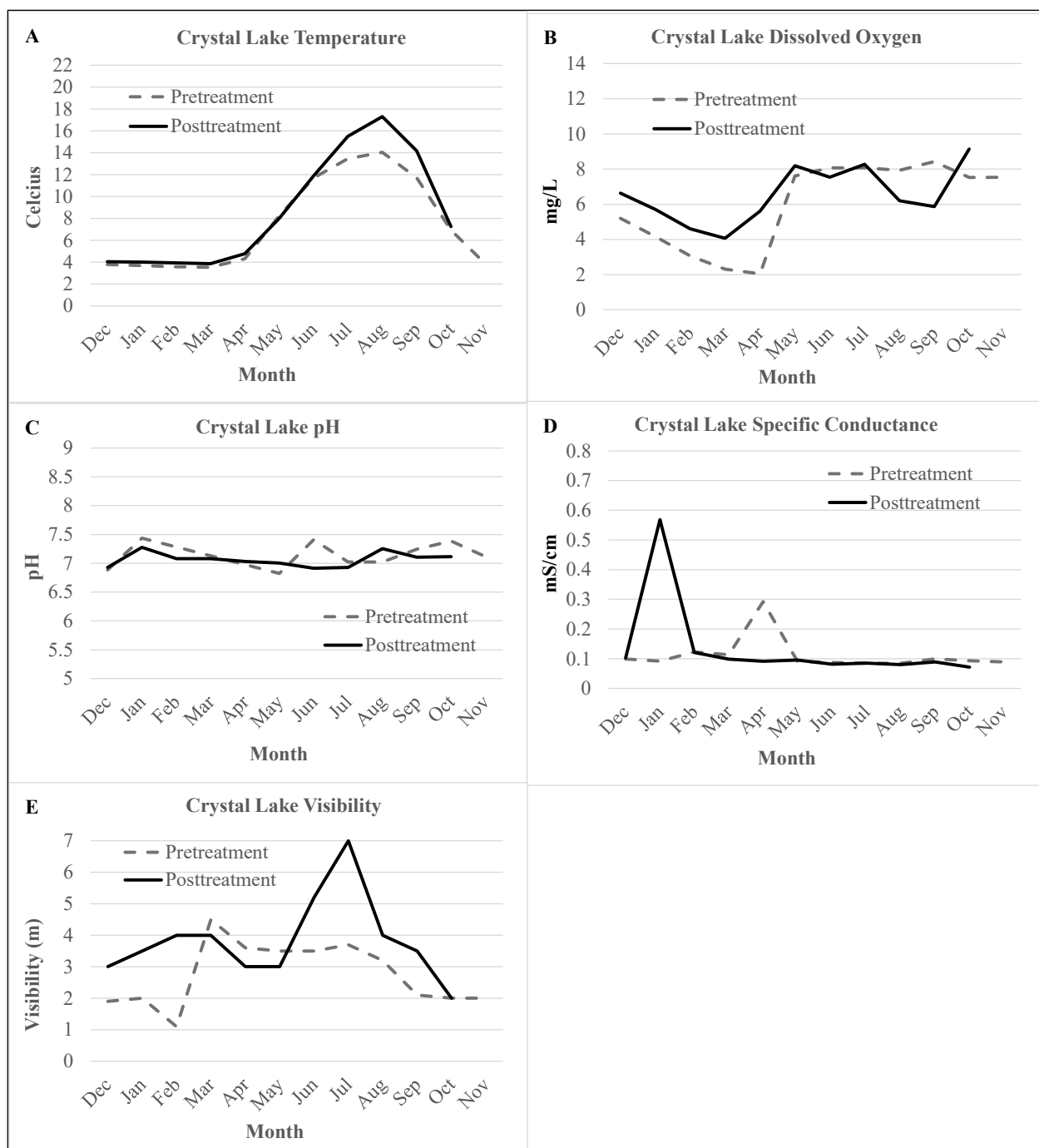


Figure 9.–Crystal Lake pretreatment (December 2016 through November 2017) and posttreatment (October 2018 through September 2019) average monthly water quality data.

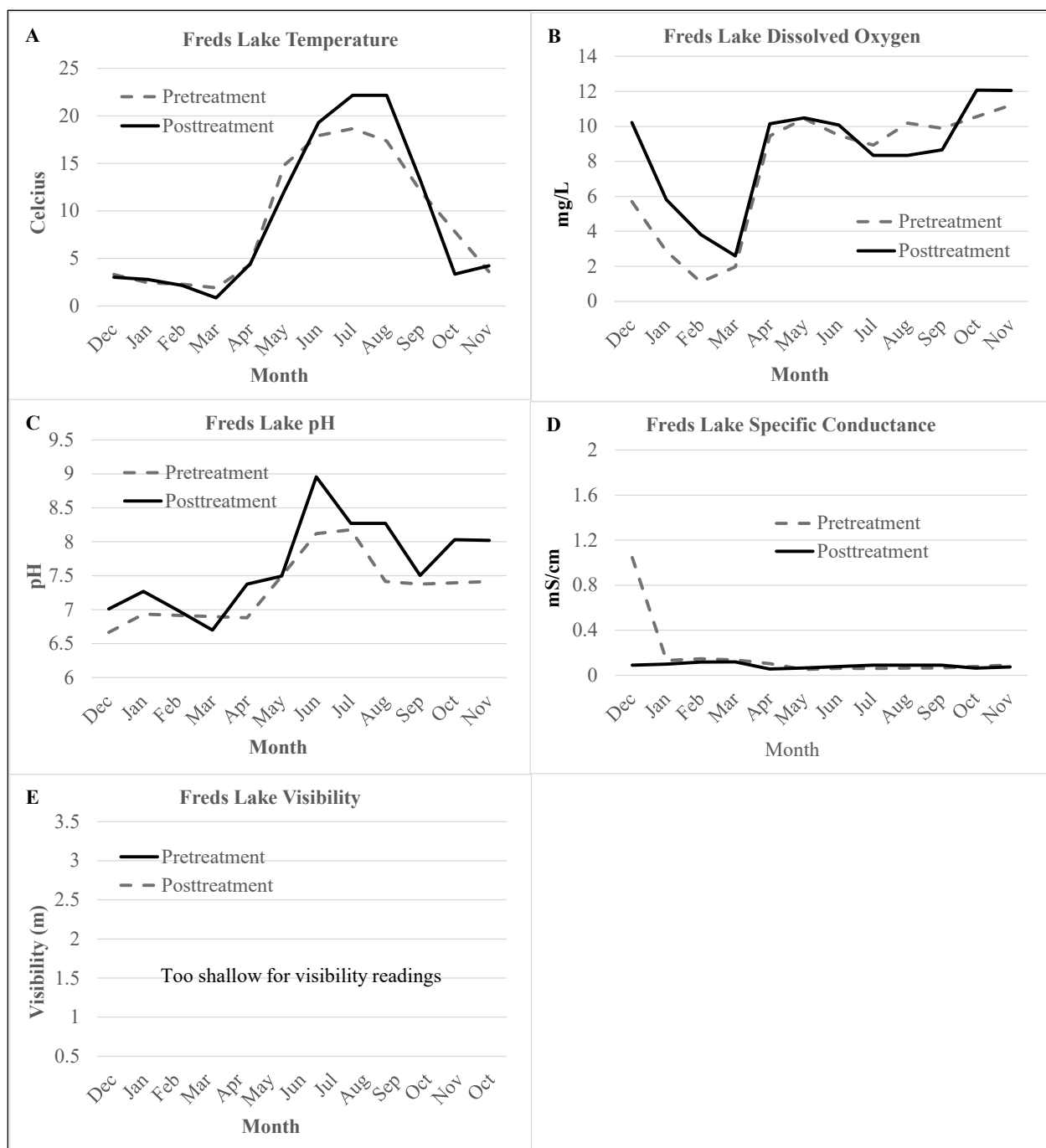


Figure 10.–Freds Lake pretreatment (December 2016 through November 2017) and posttreatment (October 2018 through September 2019) average monthly water quality data.

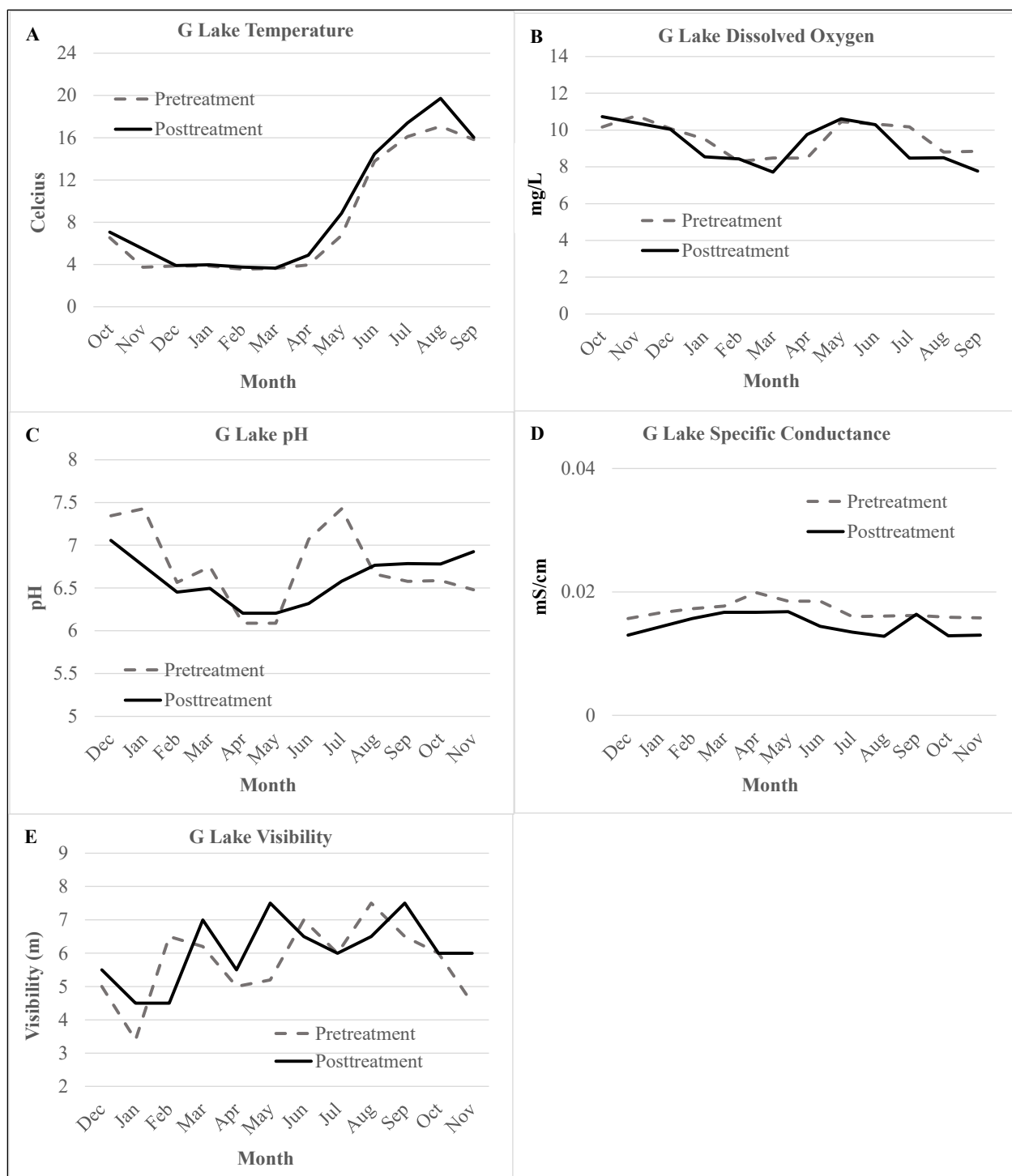


Figure 11.—G Lake pretreatment (Oct 2017 through September 2018) and posttreatment (October 2018 through September 2019) average monthly water quality data.



Figure 12.–Hope Lake pretreatment (December 2016 through November 2017) and posttreatment (October 2018 through September 2019) average monthly water quality data.

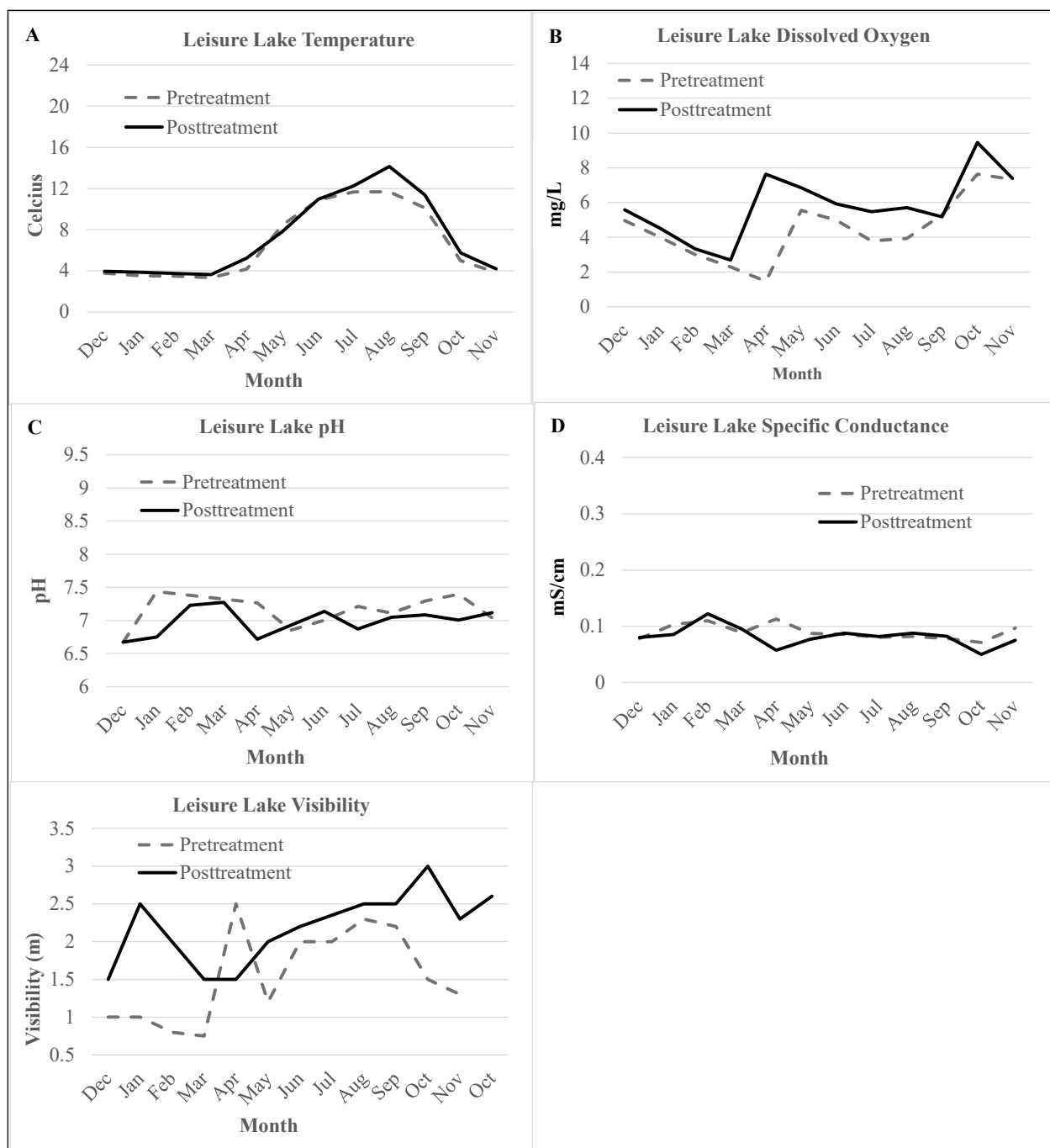


Figure 13.—Leisure Lake pretreatment (December 2016 through November 2017) and posttreatment (October 2018 through September 2019) average monthly water quality data.

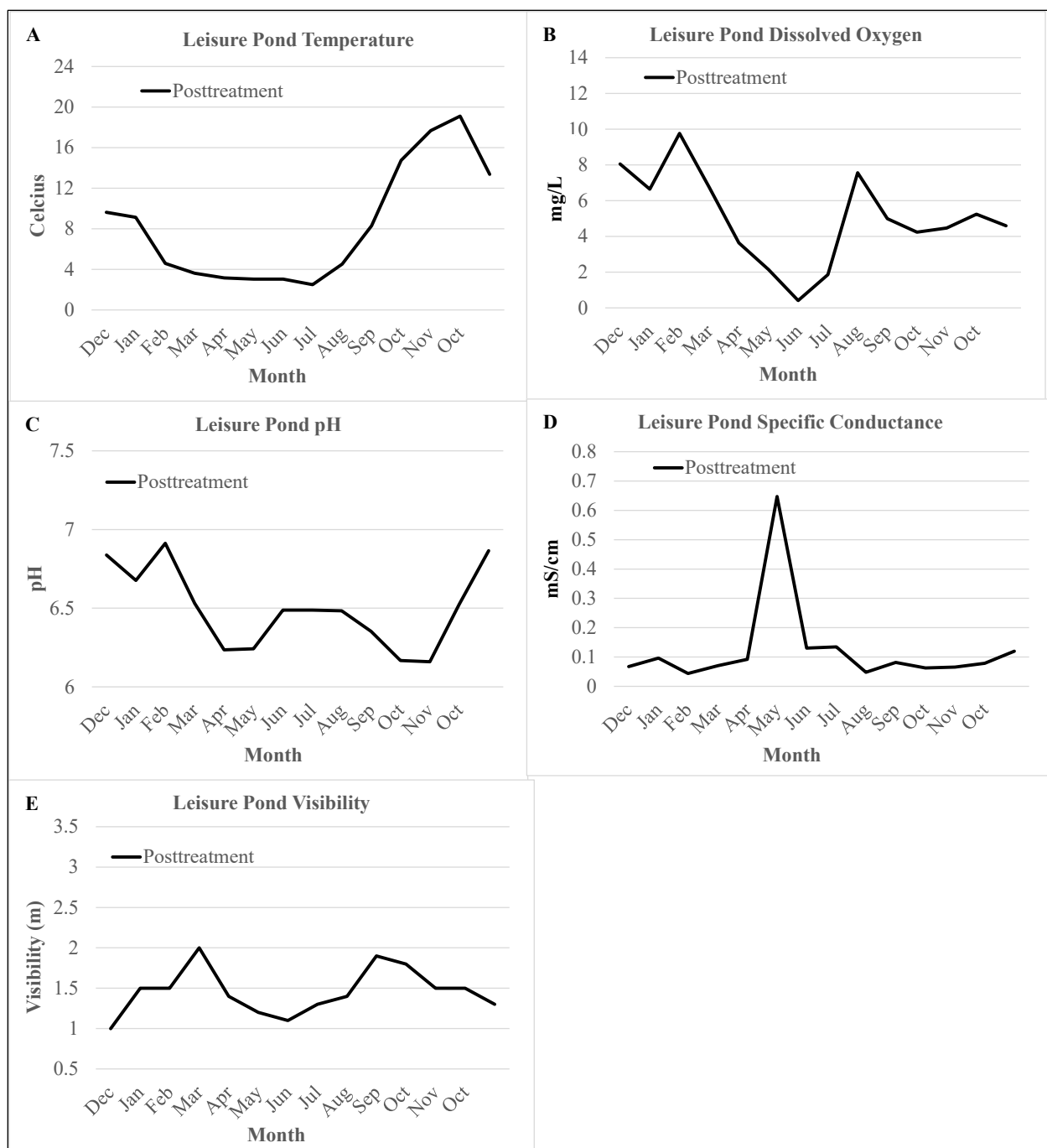


Figure 14.—Leisure Pond posttreatment (October 2018 through September 2019) average monthly water quality data.



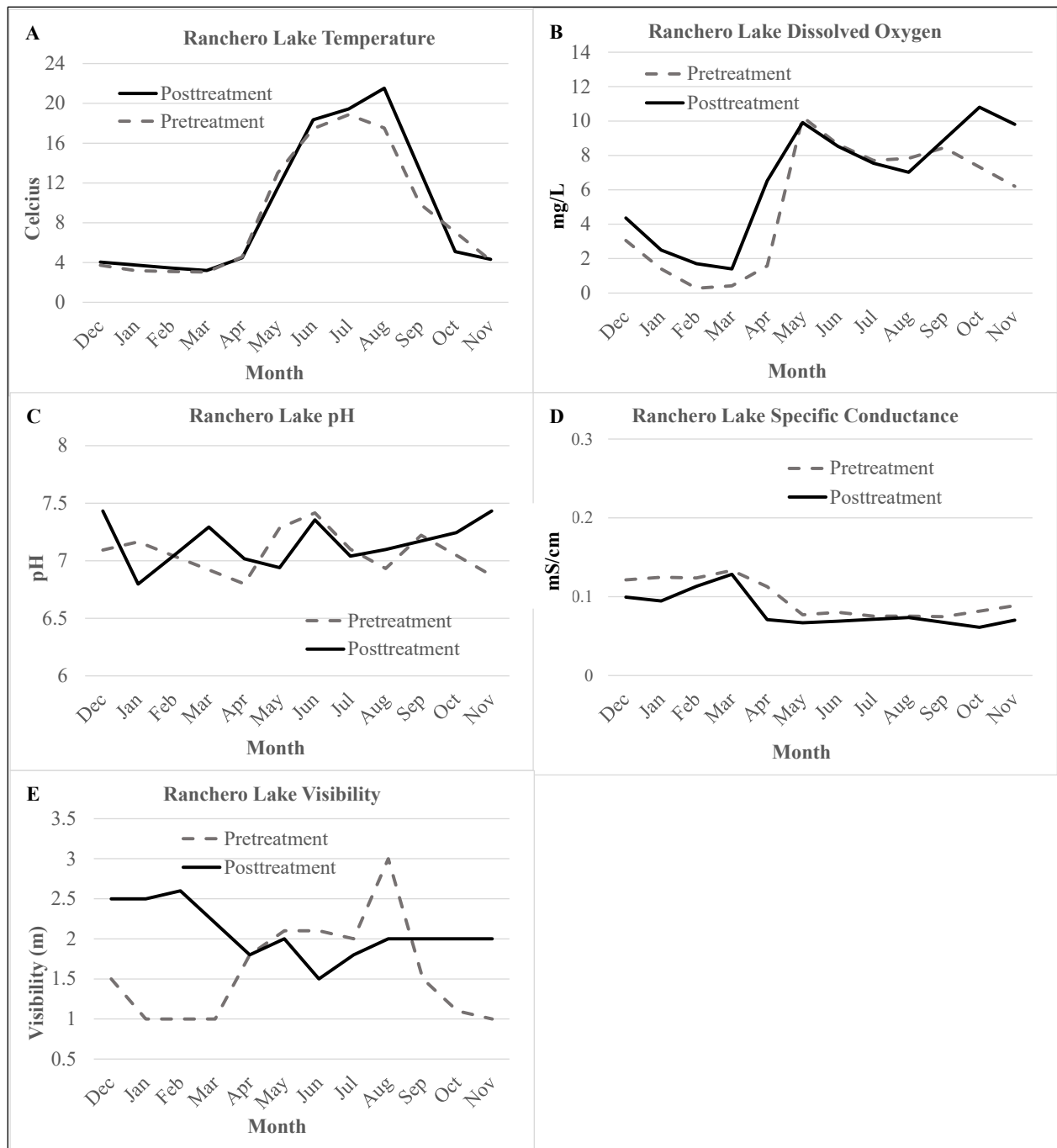


Figure 15.—Ranchero Lake pretreatment (December 2016 through November 2017) and posttreatment (August 2019) average monthly water quality data.

### eDNA, Gillnet, and Minnow Trap Surveys

Five lakes and ponds initially planned for eDNA, gillnet, and minnow trap surveys were omitted after site visits determined they were only closed marshes and too shallow to support fish.

### ***eDNA***

A northern pike eDNA survey was completed in the summer of 2017 that encompassed all waters deemed capable of supporting fish within a 1.5-mile radius of Hope Lake (Figure 16). Although it lies just beyond the 1.5-mile radius from Hope Lake, Orphe Lake was included in this survey because an unsubstantiated rumor of an angler catching a northern pike there made it suspect. Six lakes within the TRL were already known to support northern pike (CC Lake, Crystal Lake, Freds Lake, Hope Lake, Leisure Lake, and Ranchero Lake), so those waters were not included in the survey. The eDNA survey sampled 33 lakes and ponds resulted in the collection of 215 individual eDNA samples. The estimated probabilities of detecting a northern pike population of at least 20 individuals varied based on sampling intensity. Realized detection probabilities ranged from 60 to >90%, with 26 lakes (81%) exceeding 90%. Leisure Pond was the only waterbody where northern pike eDNA was detected (Table 3). Of the 5 eDNA samples collected from Leisure Pond, all tested positive for northern pike eDNA.

### ***Gillnet***

The same TRL area waters surveyed for northern pike eDNA, including Orphe Lake, were surveyed with gillnets later in the summer or early fall, except for Powers Lake where lake access was not granted for gillnetting, although eDNA sampling was allowed. The gillnet survey was later expanded to include 2 new areas outside the TRL area in response to new unconfirmed reports of northern pike catches.

A total of 7,668 hours of gillnet soak effort was expended amongst all surveyed lakes in 2017, of which 2,184.4 hours were expended in the TRL area and Orphe Lake. Of the 33 TRL area waters surveyed with gillnets, 24 were netted with enough intensity to provide an estimated >90% detection probability if a population of 20 northern pike was present. Of 9 lakes where the gillnetting effort was insufficient to achieve an estimated 90% detection probability, the detection probability ranged from a low of about 27% (Orphe Lake) to 89% (E Lake). Of all the TRL area waters surveyed with gillnets in 2017, northern pike were only discovered in Leisure Pond (Table 4), which aligned precisely with the eDNA findings, detecting northern pike in only Leisure Pond.

At G Lake, 1 large, unusual-looking esocid fish was caught in a gillnet on 1 September 2017 and was initially assumed to be an odd-colored northern pike. Subsequent gillnetting on 26 September 2017 caught several more similar-looking fish (Figure 17). Closer anatomical examination of these fish raised suspicion they could be muskellunge. Tissue samples from these fish were submitted on 26 June 2018 to the University of Minnesota where Dr. Loren Miller and a colleague (Dr. Wes Larson, University of Wisconsin–Stevens Point) compared the microsatellite data of the G Lake fish to their regional (Midwest USA) muskellunge baseline data. This analysis confirmed these fish were muskellunge; the closest genetic matches aligned with admixed populations from lower Michigan and upper Wisconsin. Using the program STRUCTURE, which analyzes multi-locus genotype data, northern Wisconsin was determined to be the likely origin of the G Lake muskellunge.

Native salmonids detected in TRL area lakes during the gillnet survey included rainbow trout and juvenile coho salmon. Three lakes contained both rainbow trout and coho salmon, 3 lakes had only coho salmon, and 1 lake had only rainbow trout (Table 4). The salmonid captures probably represent unpermitted and illegal releases because most of these lakes are closed and do not appear capable of supporting natural salmonid reproduction. Jennifer and Ruth Lakes are exceptions

because they are linked by surface water and open to Slikok Creek, a Kenai River tributary; the salmonids there appear to be naturally occurring (Figure 16).

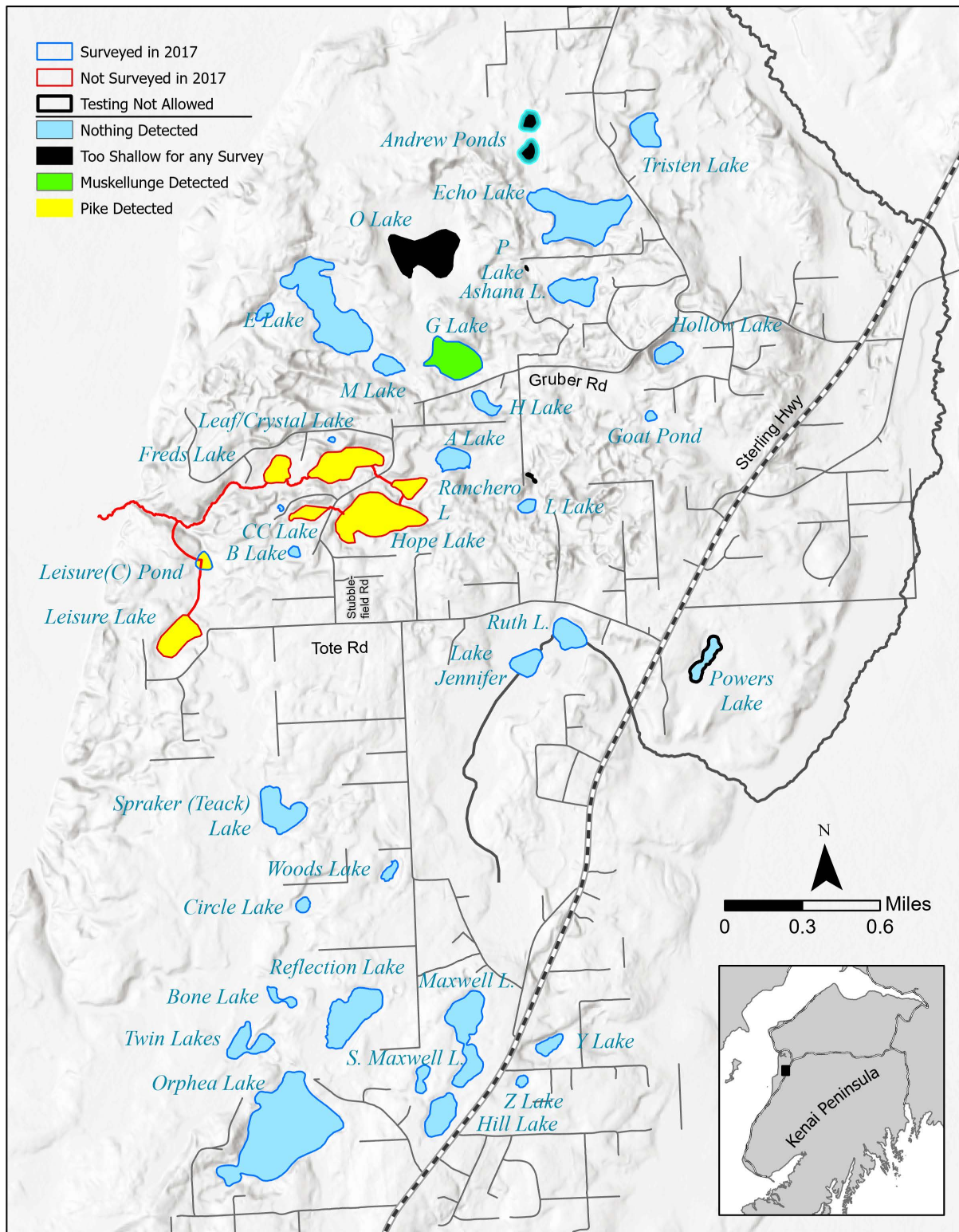


Figure 16.—Lentic waters in the Tote Road Lakes (TRL) area surveyed with gillnets and (or) eDNA sampling to confirm the distribution of northern pike.

Table 3.–Tote Road Lakes (TRL) area northern pike eDNA survey results 19 August–3 September 2017.

Collection date	Waterbody and control blank names <sup>a</sup>	Surface acres	Number of eDNA samples collected	Sampling intensity (samples/acre)	Estimated probability (%) of northern pike detection <sup>b</sup>	eDNA detection results	
						Negative	Positive
19 Jul	A Lake	5.5	5	0.91	>90	5	0
	B Lake	1	5	5.00	>90	5	0
	Pete's Lake	1.5	5	3.33	>90	5	0
	Phipps Lake	0.5	5	10.00	>90	5	0
	A, B, Pete's, and Phipps Lake Field Blank	NA	1	NA	NA	1	0
	A, B, Pete's, and Phipps Lake Travel Blank	NA	1	NA	NA	1	0
	A, B, Pete's, and Phipps Lake Lab Blank	NA	1	NA	NA	1	0
20 Jul	Ruth Lake	6.7	5	0.75	>90	5	0
	Lake Jennifer	5	5	1.00	>90	5	0
	Escape Lake	2.4	5	2.08	>90	5	0
	Goat (I) Pond	1.5	5	3.33	>90	5	0
	Ruth, Jennifer, Escape, and Goat (I) Lake Field Blank	NA	1	NA	NA	1	0
	Ruth, Jennifer, Escape, and Goat (I) Lake Field Blank	NA	1	NA	NA	1	0
	Ruth, Jennifer, Escape, and Goat (I) Lake Field Blank	NA	1	NA	NA	1	0
24 Jul	Dragon (Ashana) Lake	3	5	1.67	>90	5	0
	Hollow Lake	3.5	5	1.43	>90	5	0
	G Lake	17	5	0.29	85	5	0
	H Lake	3.2	5	1.56	>90	5	0
	Dragon, Hollow, G, and H Lake Field Blank	NA	1	NA	NA	1	0
	Dragon, Hollow, G, and H Travel Blank	NA	1	NA	NA	1	0
	Dragon, Hollow, G, and H Lake Lab Blank	NA	1	NA	NA	1	0
25 Jul	Echo Lake	30.4	6	0.20	70	6	0
	Reflection Lake	19	5	0.26	85	5	0
	Z Lake	1	5	5.00	>90	5	0
	Echo, Reflection, and Z Lake Field Blank	NA	1	NA	NA	1	0
	Echo, Reflection, and Z Travel Blank	NA	1	NA	NA	1	0
	Echo, Reflection, and Z Lab Blank	NA	1	NA	NA	1	0

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Table 3.–Page 2 of 3.

Collection date	Waterbody and control blank names <sup>a</sup>	Surface acres	Number of eDNA samples collected	Sampling intensity (samples/acre)	Estimated probability (%) of northern pike detection <sup>b</sup>	eDNA detection results	
						Negative	Positive
26 Jul	Leisure Pond	1.3	5	3.85	>90	0	5
	Orphea Lake	66	14	0.21	78	14	0
	Leisure Lake and Orphea Lake Field	NA	1	NA	NA	1	0
	Leisure Lake and Orphea Lake Travel	NA	1	NA	NA	1	0
	Leisure Lake and Orphea Lake Lab	NA	1	NA	NA	1	0
27 Jul	K Lake	1.7	5	2.94	>90	5	0
	Maxwell Lake	18.5	5	0.27	86	5	0
	Hill Lake	10	5	0.50	>90	5	0
	Tristan	7	5	0.71	>90	5	0
	K, Maxwell, Hill, and Tristan Lake Field	NA	11	NA	NA	11	0
	K, Maxwell, Hill, and Tristan Lake Travel	NA	1	NA	NA	1	0
	K, Maxwell, Hill, and Tristan Lab	NA	1	NA	NA	1	0
31 Jul	Spraker (Teack) Lake	12	5	0.42	>90	5	0
	Woods Lake	1.5	5	3.33	>90	5	0
	Circle Lake	1.6	5	3.13	>90	5	0
	Powers Lake	5.1	5	0.98	>90	5	0
	Spraker (Teack), Woods, Circle, and Powers Lake Field	NA	1	NA	NA	1	0
	Spraker (Teack), Woods, Circle, and Powers Lake Travel	NA	1	NA	NA	1	0
	Spraker (Teack), Woods, Circle, and Powers Lake Lab	NA	1	NA	NA	1	0
1 Aug	Twin Lake	8	5	0.63	>90	5	0
	Bone Lake	3.3	5	1.52	>90	5	0
	Hedge's Lake	0.25	5	20.00	>90	5	0
	Twin, Bone Hedge's Lake Field	NA	1	NA	NA	1	0
	Twin, Bone Hedge's Lake Travel	NA	1	NA	NA	1	0
	Twin, Bone Hedge's Lake Lab	NA	1	NA	NA	1	0

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Table 3.–Page 3 of 3.

Collection date	Waterbody and control blank names <sup>a</sup>	Surface acres	Number of eDNA samples collected	Sampling intensity (samples/acre)	Estimated probability (%) of northern pike detection <sup>b</sup>	eDNA detection results	
						Negative	Positive
2 Aug	South Maxwell Lake	2.5	5	2.00	>90	5	0
	Y Lake	3.25	5	1.54	>90	5	0
	South Maxwell and Y Lake Field Blank	NA	1	NA	NA	1	0
	South Maxwell and Y Travel Blank	NA	1	NA	NA	1	0
	South Maxwell and Y Blank	NA	1	NA	NA	1	0
3 Aug	E Lake	36	5	0.14	60	5	0
3 Aug	N Lake	1.8	5	2.78	>90	5	0
3 Aug	M Lake	3.5	5	1.43	>90	5	0
	E, N, and M Lake Field	NA	1	NA	NA	1	0
	E, N, and M Lake Travel	NA	1	NA	NA	1	0
	E, N, and M Lake Lab	NA	1	NA	NA	1	0

*Note:* All eDNA samples were collected individually in sterile 1 L bottles and analyzed for northern pike DNA by the USFWS Conservation Genetics Lab in Anchorage, AK.

<sup>a</sup> Travel, field, and lab blanks served as control samples to detect eDNA contamination during various stages of sample handling and were composed of purely deionized water. Each sample blank type (field, travel, lab) was handled like all the other samples.

<sup>b</sup> The estimated probability of detecting a population of 20 northern pike based on surface acreage and number of eDNA samples.



Figure 17.—Muskellunge collected from G lake in 2017.

Table 4.–Gillnet survey effort and catch data in Kenai Peninsula waters suspected to have introduced northern pike populations, 2017.

Area	Waterbody <sup>a,b</sup>	Surf. acres	Latitude	Longitude	Net set date	No. nets fished	Total netting effort (h)	Est. prob. of detection (%) <sup>c</sup>	Catch					
									Rain- bow trout	Adult coho salmon	Juv. coho salmon	Northern pike	Muskel -lunge	Long- nose sucker
Coho Loop	Big Logs	1.5	60.295207	-151.350657	19 Sep	2	46.4	>90	0	0	0	0	0	0
	House Pond													
	Bill Toombs L.	6.4	60.296175	-151.345621	18 Sep	4	87.4	>90	0	0	0	0	0	0
	Fish and Game L.	36.6	60.291935	-151.325848	19 Sep	6	137.4	>77	75	0	0	0	0	0
	Hackney L.	8.8	60.291488	-151.352681	19 Sep	6	32.3	>90	43	0	0	0	0	0
	Kadaca (Seth) L.	11.9	60.280244	-151.342700	15 Sep	12	49.6	>90	0	0	0	0	0	0
	Kingsley L.	37.5	60.288010	-151.346872	18 Sep	10	47.0	>39	13	0	0	0	0	0
	Mike1 L.	3.6	60.291012	-151.335038	18 Sep	4	16.5	>83	11	0	0	0	0	0
	Mike1 L.	3.6	60.291012	-151.335038	19 Sep	4	17.9	>90	7	0	0	0	0	0
	Mike2 L.	1.3	60.292946	-151.336378	18 Sep	4	9.2	>90	5	0	0	0	0	0
	Mike2 L.	1.3	60.292946	-151.336378	19 Sep	4	20.3	>90	3	0	0	0	0	0
	North Bottleneck L.	23.5	60.285589	-151.360051	14 Sep	10	51.1	>58	69	0	0	0	0	0
	Forbaugh L.	11.3	60.297906	-151.373460	26 Sep	10	58.1	>87	1	0	0	0	0	0
	South Bottleneck L.	11.3	60.281401	-151.364237	14 Sep	10	49.7	>82	46	0	0	0	0	0
	Warfle's L.	7.7	60.290116	-151.365627	18 May	12	48.0	>90	0	0	0	6	0	0
	Warfle's L.	7.7	60.290116	-151.365627	16 Oct	12	4,327.5	>90	0	0	0	0	0	0
						Subtot.	4,998.4	NA	273	0	0	6	0	0
Swanson River (Crane Lake drainage)	Crane L.	53.6	60.791754	-150.955320	17 Aug	6	143.1		10	0	1	0	0	0
	Crane L. (East)	37.6	60.783255	-150.990736	17 Aug	4	86.9		2	4	0	0	0	0
	Crane L. (West)	35.2	60.778841	-150.980816	17 Aug	4	85.1		37	0	18	0	0	21
	Snipe L.	95.9	60.757245	-150.974740	17 Aug	10	170.6		103	0	37	0	0	0
						Subtot.	485.7	NA	152	4	56	0	0	21

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Table 4.–Page 2 of 3.

Area	Waterbody <sup>a,b</sup>	Surf. acres	Latitude	Longitude	Net set date	No. nets fished	Total netting effort (h)	Est. prob. of detection (%) <sup>c</sup>	Catch					
									Rain- bow trout	Adult coho salmon	Juv. coho salmon	Northern pike	Muskel -lunge	Long- nose sucker
TRL	A Lake	5.5	60.423844	-151.179928	6 Sep	12	330.7	>90	0	0	0	0	0	0
	B Lake	1	60.419439	-151.196186	6 Sep	2	50.1	>90	0	0	0	0	0	0
	Bone L.	3.3	60.395620	-151.201932	11 Sep	3	66.5	>90	0	0	0	0	0	0
	Circle L.	1.6	60.399855	-151.196870	11 Sep	3	64.2	>90	0	0	0	0	0	0
	Dragon (Ashana) L.	3	60.433102	-151.165759	9 Aug	10	48.3	>90	2	0	0	0	0	0
	E Lake	36	60.435090	-151.195706	12 Sep	10	202.2	>89	0	0	0	0	0	0
	Echo L.	30.4	60.437233	-151.162258	9 Aug	10	49.3	>47	0	0	0	0	0	0
	Escape L.	2.4	60.427669	-151.195803	13 Oct	3	43.6	>90	0	0	0	0	0	0
	G Lake	17	60.428991	-151.176984	1 Sep	11	50.7	>90	0	0	0	0	1	0
	G Lake	17	60.428991	-151.176984	26 Sep	6	103.0	>90	0	0	0	0	3	0
	Goat (I) Pond	1.5	60.426259	-151.155895	8 Sep	8	39.0	>90	0	0	1	0	0	0
	H Lake	3.2	60.427445	-151.172956	30 Aug	10	49.3	>90	0	0	0	0	0	0
	Hedge's L.	0.25	60.425682	-151.191848	21 Aug	3	70.3	>90	0	0	0	0	0	0
	Hill L.	10	60.388909	-151.183254	15 Aug	10	50.7	>90	0	0	0	0	0	0
	Hollow L.	3.5	60.429427	-151.153532	10 Aug	10	54.4	>90	0	0	0	0	0	0
	K Lake	1.7	60.421854	-151.169364	31 Aug	10	46.2	>90	0	0	0	0	0	0
	Lake Jennifer	5	60.413280	-151.169386	5 Sep	4	12.7	>63	14	0	30	0	0	0
	Leisure Pond	1.3	60.418927	-151.208101	5 Sep	3	61.6	>90	0	0	0	5	0	0
	M Lake	3.5	60.430424	-151.185806	12 Sep	2	41.6	>90	10	0	22	0	0	0
	Maxwell L.	18.5	60.392478	-151.179421	8 Aug	10	43.1	>60	0	0	1	0	0	0
	N Lake	1.8	60.432899	-151.198270	12 Sep	2	40.4	>90	0	0	0	0	0	0
	Orphea L. <sup>d</sup>	66	60.389616	-151.203652	8 Aug	10	53.0	>27	0	0	0	0	0	0
	Petes L.	1.5	60.426542	-151.205991	7 Sep	10	45.9	>90	0	0	0	0	0	0
	Phipps L.	0.5	60.422088	-151.197837	13 Oct	3	71.3	>90	0	0	0	0	0	0
	Reflection L.	19	60.393653	-151.194046	7 Aug	10	49.2	>64	0	0	0	0	0	0
	Ruth L.	6.7	60.413665	-151.163125	23 Aug	4	17.1	>63	5	0	1	0	0	0

-continued-

Table 4.–Page 3 of 3.

Area	Waterbody <sup>a,b</sup>	Surf. acres	Latitude	Longitude	Net set date	No. nets fished	Total netting effort (h)	Est. prob. of detection (%) <sup>c</sup>	Catch					
									Rain- bow trout	Adult coho salmon	Juv. coho salmon	Northern pike	Muskel -lunge	Long- nose sucker
TRL (continued)	S. Maxwell L.	2.5	60.389437	-151.185611	15 Aug	10	52.5	>90	0	0	0	0	0	0
	Spraker (Teack) L.	12	60.404681	-151.197264	14 Aug	10	49.9	>81	0	0	7	0	0	0
	Tristan L.	7	60.443158	-151.155316	1 Sep	10	53.4	>90	0	0	0	0	0	0
	Twin L.	8	60.392287	-151.201512	11 Sep	6	133.9	>90	0	0	0	0	0	0
	Woods L.	1.5	60.402004	-151.187418	16 Aug	10	50.4	>90	0	0	0	0	0	0
	Y Lake	3.25	60.391257	-151.171541	22 Aug	10	52.8	>90	0	0	0	0	0	0
	Z Lake	1	60.389695	-151.173677	29 Aug	6	36.9	>90	0	0	0	0	0	0
Subtot.							2,184.4		31	0	62	5	4	0
Grand total							7,668.4		456	4	118	11	4	21

<sup>a</sup> Many waterbody names are unofficial.

<sup>b</sup> Some lakes are listed twice because they were netted more than once on nonconsecutive days.

<sup>c</sup> See Appendix A2 for calculations to estimate the probability of detecting a population of 20 northern pike from gillnetting efforts.

<sup>d</sup> Orphea Lake is located outside the 1.5-mile radius of Hope Lake constituting the TRL area survey area; it was surveyed due to an unsubstantiated report that northern pike may exist there.

In the spring of 2017, ADF&G received an unsolicited report of northern pike caught by an angler at Warfle Lake, a 7-acre landlocked lake in the Coho Loop Road area in Kasilof. Also, a different anonymous report was received about anglers catching northern pike at an unnamed lake north of the Swanson River and next to a recently built gas-exploration ice-road in the Kenai National Wildlife Refuge (KNWR). This description loosely matched the description for the Crane Lake system, a tributary of the Swanson River (Figure 18).

Both northern pike reports prompted an expansion of the TRL-area gillnet survey to include Warfle Lake in Kasilof and 4 lakes within the Crane Lake system. On May 17, ADF&G gillnetted Warfle Lake and caught multiple northern pike. This prompted an expanded gillnet survey of the area and 11 more lakes deemed susceptible to northern pike due to their proximity to Warfle Lake were surveyed (Figure 19). No northern pike were detected in the expanded survey area near Warfle Lake, although rainbow trout were caught in 8 of the 11 lakes netted under the expanded survey.

No northern pike were caught in the Crane Lake drainage during gillnetting that occurred between 17 and 18 August 2017, although rainbow trout and longnose suckers were caught (Table 4).

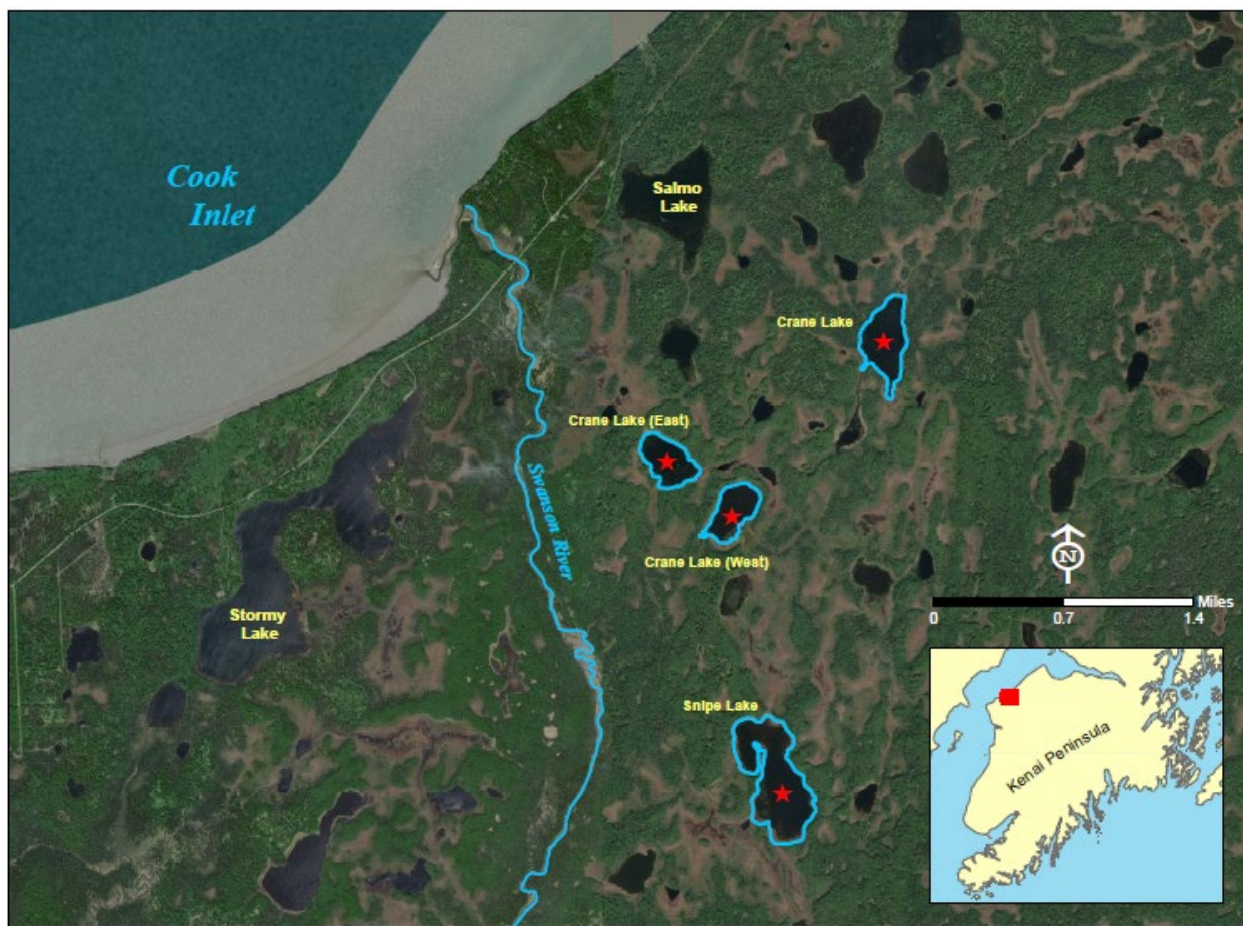


Figure 18.—Crane Lake drainage (Swanson River tributary) gillnet survey area, red stars denote surveyed lakes, 2017.

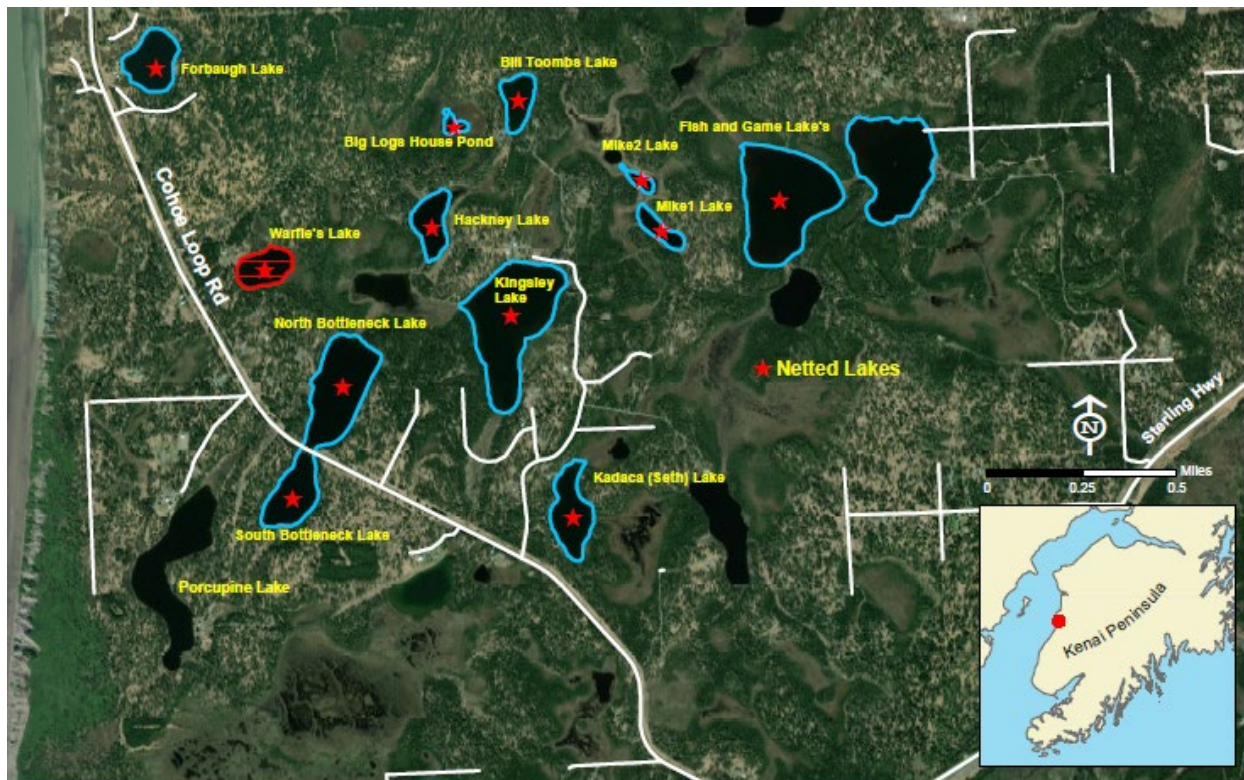


Figure 19.—Coho Loop Road area gillnet survey area (Kasilof), red stars denote surveyed lakes, red hash lines identify Warfle Lake where northern pike were discovered in 2017.

### *Minnow Trap*

In 2017, minnow trapping surveys were conducted in most waters surrounding the TRL area within 1.5 miles of Hope Lake and at select lakes in the Warfle Lake–Coho Loop area. Minnow trapping was not conducted at the 4 lakes surveyed with gillnets in the Crane Lake system (Swanson River drainage). Eight lakes that are part of the TRL area were not surveyed with minnow traps by ADF&G in 2017, but they were surveyed by McGill University researchers in 2018.

A total of 1,515 hours of minnow trap soak effort was expended between all lakes surveyed by ADF&G in 2017 (Table 5). No northern pike were captured in minnow traps; however, threespine stickleback were detected in 14 of the 31 lakes surveyed in the TRL area. Rainbow trout were captured by minnow traps at M Lake but nowhere else in the TRL area, and no coho salmon were captured by minnow trap in any TRL location. Additionally, McGill University researchers involved with the TRL threespine stickleback releases conducted their own minnow trap survey during 2018 in 8 TRL waterbodies where esocids had been captured with gillnets (Table 6). This minnow trapping only caught threespine stickle at G Lake, and no other species were caught in the 7 other lakes.

In the Warfle Lake–Coho Loop survey area, minnow trapping caught rainbow trout in 2 lakes and threespine stickleback were caught in all lakes but Warfle Lake; it is possible threespine stickleback were eradicated by northern pike predation because lake residents reported they were present there prior to northern pike introduction.

Table 5.—Minnow trapping survey and catch data for lakes near the Tote Road Lakes (TRL) and Coho Loop Road areas, 2017.

Area	Waterbody <sup>a</sup>	Trap check date	Sum of effort (h)	Northern pike	Dolly Varden	Coho salmon	Rainbow trout	Threespine stickleback
Coho Loop	Big Logs House Pond	20 Sep	116.8	0	0	0	0	20
	Bill Toombs Lake	19 Sep	114.5	0	0	0	0	29
	Fish and Game Lake	20 Sep	15.3	0	0	0	25	88
	Hackney Lake	Did not trap	—	—	—	—	—	—
	Kadaca (Seth) Lake	15 Sep	19.6	0	0	0	0	63
	Kingsley Lake	18 Sep	22.5	0	0	0	0	213
	Mike1 Lake	18 Sep	18.7	0	0	0	0	18
	Mike2 Lake	19 Sep	24.9	0	0	0	0	25
	North Bottleneck Lake	14 Sep	21.7	0	0	0	2	13
	Forbaugh Lake	26 Sep	25.8	0	0	0	0	22
	South Bottleneck Lake	14 Sep	24.0	0	0	0	0	74
	Warfle's Lake	18 May	16.0	0	0	0	0	0
	Subtotal		419.8	0	0	0	27	565
TRL	A Lake	6 Sep	13.2	0	0	0	0	64
	B Lake	7 Sep	13.1	0	0	0	0	0
	Bone Lake	12 Sep	110.5	0	0	0	0	113
	Circle Lake	12 Sep	106.8	0	0	0	0	0
	Dragon (Ashana) Lake	9 Aug	13.9	0	0	0	0	0
	E Lake	13 Sep	98.3	0	0	0	0	27
	Echo Lake	9 Aug	10.5	0	0	0	0	11
	Escape Lake	Did not trap	—	—	—	—	—	—
	G Lake	1 Sep	13.6	0	0	0	0	8
	Goat (I) Pond	8 Sep	20.5	0	0	0	0	0
	H Lake	30 Aug	16.3	0	0	0	0	0
	Hedge's Lake	22 Aug	47.0	0	0	0	0	0
	Hill Lake	15 Aug	6.4	0	0	0	0	0
	Hollow Lake	10 Aug	24.0	0	0	0	0	1
	K Lake	31 Aug	18.1	0	0	0	0	0
	Lake Jennifer	5 Sep	7.5	0	0	0	0	27
	Leisure Pond	6 Sep	102.1	0	0	0	0	0
	M Lake	13 Sep	99.9	0	0	0	11	28

-continued-

Table 5.–Page 2 of 2.

Area	Waterbody <sup>a</sup>	Trap check date	Sum of effort (h)	Northern pike	Dolly Varden	Coho salmon	Rainbow trout	Threespine stickleback
TRL (continued)	Maxwell Lake	8 Aug	6.2	0	0	0	0	0
	N Lake	13 Sep	100.4	0	0	0	0	0
	Orphea Lake	8 Aug	8.6	0	0	0	0	1
	Pete's Lake	7 Sep	17.9	0	0	0	0	0
	Phipps Lake	22 Aug	47.1	0	0	0	0	0
	Reflection Lake	7 Aug	11.5	0	0	0	0	36
	Ruth Lake	23 Aug	11.4	0	0	0	0	139
	S. Maxwell Lake	15 Aug	13.3	0	0	0	0	0
	Spraker (Teack) Lake	14 Aug	12.4	0	0	0	0	28
	Tristan Lake	1 Sep	5.9	0	0	0	0	98
	Twin Lake	12 Sep	110.8	0	0	0	0	19
	Woods Lake	16 Aug	8.5	0	0	0	0	0
	Y Lake	22 Aug	12.7	0	0	0	0	0
	Z Lake	29 Aug	7.1	0	0	0	0	0
	Subtotal		1,095.4	0	0	0	11	600
Grand total			1,515.2	0	0	0	38	1,165

<sup>a</sup> Many waterbody names are unofficial.



Table 6.—Tote Road Lakes area pretreatment minnow trapping survey and catch data, 3–5 June 2018.

Treatment status	Location	Date	Number of traps fished	Total trapping effort (h) <sup>a,b</sup>	Threespine stickleback	Northern pike	Rainbow trout	Coho salmon
Pretreatment	CC Lake	5 Jun	20	104.04	0	0	0	0
	Crystal Lake	5 Jun	20	40.00	0	0	0	0
	Freds Lake	9 Jun	20	61.97	0	0	0	0
	G Lake	2 Jun	3	9.00	95	0	0	0
	Hope Lake	4 Jun	20	58.17	0	0	0	0
	Leisure Lake	3 Jun	2	6.00	0	0	0	0
	Leisure Pond	3 Jun	20	65.63	0	0	0	0
	Ranchero Lake	4 Jun	20	45.72	0	0	0	0
Total			125	390.52	95	0	0	0

<sup>a</sup> The minnow trap surveys were conducted by researchers under the supervision of Dr. Andrew Hendry, University of McGill, Quebec, Canada.

<sup>b</sup> Minnow traps were cylindrical with funnel openings at both ends and constructed with one-quarter-inch mesh galvanized screen.

## **Invasive Fish Sampling and Salvage**

Although not a task directly associated with this project, ADF&G used gillnets to remove and sample 192 northern pike from Ranchero Lake on 8 May 2018. These fish were collected for tissue samples (fin clips) as requested by the ADF&G Gene Conservation Lab for developing northern pike genetic sex markers. Stomachs from these fish were collected and preserved in cold storage at the request of researchers from McGill University (Quebec, Canada), who are involved with the reintroduction of threespine stickleback. All the northern pike were sampled for sex and length (FL).

From 15 August 2018 until 20 September 2018, 5 TRL waterbodies were gillnetted to salvage northern pike for food donation and to reduce nuisance issues due to excessive fish carcasses following the planned rotenone treatment (e.g., odors, animal scavenging). These lakes included CC Lake, Crystal Lake, Hope Lake, Leisure Lake, and Ranchero Lake. The northern pike and muskellunge populations in the other 3 lakes in the TRL area were deemed too small to warrant a salvage effort. A total of 2,020 hours of netting effort was expended among the 5 lakes, and 287 northern pike were removed (Table 7).

Most fish of practical size for food salvage (>300 mm) were donated to area residents, and others were utilized for educational purposes. The salvage goal was to continue daily netting at each lake until the daily catch fell below 50% of the first day's catch. This removal goal was met at CC Lake, Crystal Lake, and Hope Lake, but not at Leisure Lake and Ranchero Lake where the last day's catches represented 54% and 92% of the first day's catches, respectively.

Nearly all TRL northern pike collected for food salvage and tissue sampling in 2018, and all muskellunge collected in 2017 and 2018, were measured for length (FL). Many of these fish were also examined to identify sex via dissection. TRL northern pike data were combined to calculate descriptive statistics for FL and sex ratios. A total of 479 northern pike were measured for FL in 2018 (Table 8, Figure 20, Appendix E1). Mean length was 359 mm (SD = 88 mm), with a length range of 825 mm to 183 mm. A total of 305 northern pike were identified for sex, 69% ( $N = 210$ ) were male and 31% ( $N = 95$ ) female. Of the males, mean FL was 378 mm (SD 82 mm) and ranged from 209 mm to 616 mm. Of the females, mean FL was 379 mm (SD = 105 mm) and ranged from 211 mm to 825 mm.

A total of 8 muskellunge were removed from G Lake during 2017 and 2018. Six were caught with gillnets, 1 was caught angling, and another recovered dead following the rotenone treatment. FL was recorded for 7 muskellunge. Mean muskellunge FL was 669 mm (SD = 145 mm) and ranged from 795 mm to 405 mm (Table 8).



Table 7.—Pretreatment northern pike detection and salvage gillnetting efforts in the Tote Road Lakes (TRL), 2006 and 2018.

Location	Survey Type <sup>a</sup>	Net set date (M/D/Y)	Net pull date (M/D/Y)	Number of nets fished	Hours of netting effort	Number of fish captured	
						Northern pike	Muskellunge
CC Lake	Detection	6/8/2006	6/9/2006	1	22.8	3	0
	Salvage	8/15/2018	8/15/2018	16	66.0	19	0
	Salvage	8/16/2018	8/16/2018	16	82.5	16	0
	Salvage	8/20/2018	8/20/2018	16	73.6	9	0
	Subtotal			49	244.9	47	0
Crystal Lake	Detection	6/8/2006	6/9/2006	2	48.8	20	0
	Salvage	8/21/2018	8/21/2018	20	82.6	16	0
	Salvage	8/22/2018	8/22/2018	20	82.6	21	0
	Salvage	8/23/2018	8/23/2018	22	122.4	19	0
	Salvage	8/27/2018	8/27/2018	20	82.4	30	0
	Salvage	8/28/2018	8/28/2018	22	138.1	16	0
	Salvage	8/29/2018	8/29/2018	18	107.0	6	0
	Subtotal			124	663.8	128	0
Freds Lake	Detection	6/14/2006	6/15/2006	1	24.7	5	0
G Lake	Detection	9/1/2017	9/1/2017	11	50.7	0	1
	Detection	9/26/2017	9/27/2017	6	103.0	0	3
	Detection	6/19/2018	6/19/2018	16	80.0	0	3
	Subtotal			33	233.7	0	7
Hope Lake	Detection	6/8/2006	6/9/2006	1	22.5	42	0
	Salvage	10/2/2018	10/2/2018	27	107.4	25	0
	Salvage	10/3/2018	10/4/2018	28	654.3	11	0
	Subtotal			56	784.2	78	0
Leisure Lake	Detection	6/14/2006	6/15/2006	1	25.1	1	0
	Salvage	8/13/2018	8/13/2018	18	81.2	26	0
	Salvage	8/14/2018	8/14/2018	17	76.1	14	0
	Subtotal			35	157.3	40	0
Leisure Pond	Salvage	9/5/2017	9/6/2017	3	61.7	5	0
Ranchero Lake	Detection	6/8/2006	6/9/2006	1	22.6	31	0
	Salvage	5/8/2018	5/9/2018	14	408.0	192	0
	Salvage	9/18/2018	9/18/2018	16	85.7	25	0
	Salvage	9/19/2018	9/19/2018	18	76.4	21	0
	Salvage	9/20/2018	9/20/2018	18	76.5	23	0
	Subtotal			49	592.6	269	0
Total				137	2762.9	572	7

Note: Gillnets were made with floating hanging lines and bottom lead lines and all were 120 ft in length, 6 ft deep, and composed of 6 different monofilament mesh panels in the following sizes: 0.75 in, 1.0 in, 1.25 in, 1.5 in, 1.75 in and 2.0 in.

<sup>a</sup> “Detection” means gillnetting to determine which lakes had northern pike during 2006 under a different project; “salvage” means gillnetting to collect fish in 2017 or 2018 for food or educational use, and to reduce posttreatment dead fish nuisance issues.

Table 8.—Summary table for length and sex of northern pike and muskellunge captured during pretreatment salvage efforts, 2017–2018.

Statistic	Northern pike			Muskellunge
	All fish	Male fish	Female fish	
Total inspected for length	479	210	95	7
Mean length	359 mm	378 mm	379 mm	669
SD (length)	88 mm	82 mm	105 mm	145
Maximum length	825 mm	616 mm	825 mm	795
Minimum length	183 mm	209 mm	211 mm	405
Total identified for sex	305	210	95	0
Percent sex	100%	69%	31%	—

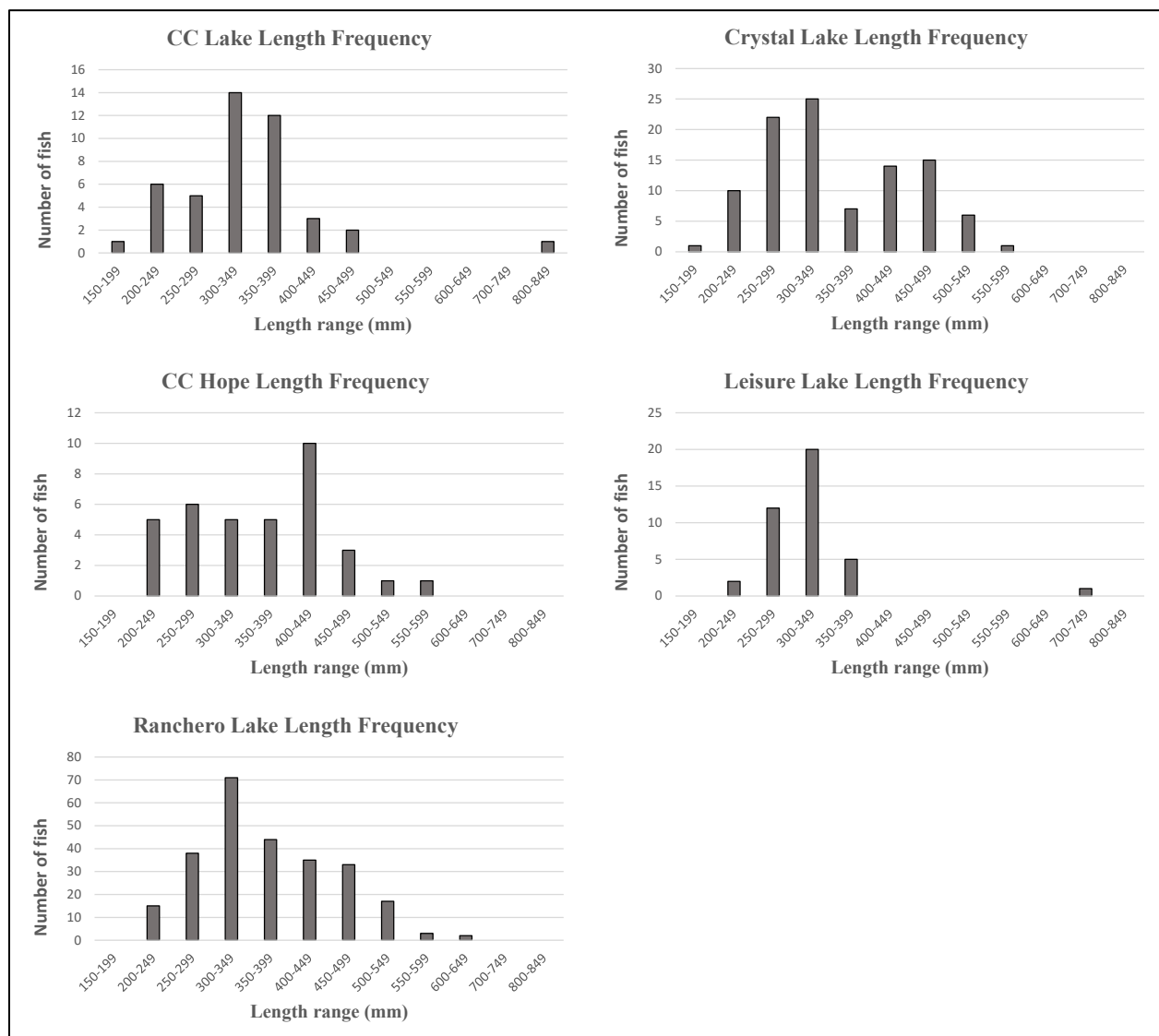


Figure 20.—Histograms of northern pike fork lengths from select Tote Road Lakes (TRL) waters, 2018.

## BIOASSAYS

Bioassays to determine the minimum effective dose (MED) of the rotenone product (CFT Legumine) were conducted at Ranchero Lake on 4 October 2018. One bioassay group tested a range of rotenone concentrations dosed in pure Ranchero Lake water; another bioassay group tested this same range of rotenone concentrations dosed in Ranchero Lake water with the addition of 1 cup of lake muck and vegetation added to each bioassay container to mimic the presence of lake organics. Organics can bind with the active ingredient (rotenone) and reduce its toxicity, so the latter bioassay would help assess that effect.

For each bioassay group, 6 juvenile coho salmon (each about 110 mm FL) were added to each of 6 plastic buckets filled with 20 L of lake water. At the time of the bioassays, the Ranchero Lake water temperature was 10.9°C, specific conductance was 0.067 mS/cm, dissolved oxygen was 8.5 mg/L, and pH was 7.2. The rotenone concentrations tested in both bioassay groups were 0.00 ppm (control), 0.125 ppm, 0.025 ppm, 0.04 ppm, 0.05 ppm, 1.0 ppm, and 2.0 ppm.

Fish in all bioassay trials with rotenone concentrations between 0.125 and 2.0 ppm died within 145 minutes or less. No fish died in the bioassays devoid of rotenone dosing (controls). In general, it took slightly longer for fish to become impaired and die in the bioassays loaded organics. The speed of rotenone exposure effects was positively associated with increasing rotenone concentrations (Table 9).

Standard operating procedures for fish eradication suggest the minimum target concentration of rotenone should be double that which achieved 100% mortality in the bioassays after 8 hours (Finlayson et al. 2010). These bioassay results indicated that all tested concentrations of rotenone, except the controls, would satisfy this guideline.

When selecting the target rotenone concentration, environmental factors present in the TRL that could affect the toxicity of rotenone included the presence of dense aquatic vegetation beds, suspended particles like algae, dilution from adjacent wetland and springs, deep organic substrate, and deep water (>15 feet) where mixing of rotenone can be inhibited. The observed potency of rotenone in the bioassays, even at relatively low concentrations, reassured us that our proposed target rotenone concentration (40 ppb) would likely be sufficient to kill northern pike even when accounting for the multiple environmental variables found in TRL waters that could attenuate its efficacy.

Table 9.—Results of rotenone bioassays, Ranchero Lake, 4 October 2018.

Time	Fish status <sup>a</sup>													
	No organic loading bioassay <sup>b</sup> rotenone concentration (ppb)							Organic loading bioassay <sup>c</sup> rotenone concentration (ppb)						
	0.0	12.5	25	40	50	100	200	0.0	12.5	25	40	50	100	200
12:15	Set up <sup>d</sup>	Set up <sup>d</sup>	—	—	—	—	—	Set up <sup>d</sup>	Set up <sup>d</sup>	—	—	—	—	—
12:17	—	—	Set up <sup>d</sup>	—	—	—	—	—	—	Set up <sup>d</sup>	—	—	—	—
12:07	—	—	—	Set up <sup>d</sup>	—	—	—	—	—	—	Set up <sup>d</sup>	—	—	—
12:20	—	—	—	—	Set up <sup>d</sup>	—	—	—	—	—	—	Set up <sup>d</sup>	—	—
12:25	—	—	—	—	—	Set up <sup>d</sup>	—	—	—	—	—	—	Set up <sup>d</sup>	—
12:29	—	—	—	—	—	—	Set up <sup>d</sup>	—	—	—	—	—	—	Set up <sup>d</sup>
12:40	6A	6A	6A	6I	6I	5R, 1A	4R	6A	6A	6A	2A, 4I	3A, 3I	4I, 2R	4A, 2I
12:45	6A	6A	1I, 5A	6I	6I	6R	6R	6A	6A	6A	2A, 4I	3A, 3I	2I, 4R	2A, 4I
12:50	6A	6A	2R, 1I, 3A	6R	6I	6R	6R	6A	5A, 1I	6A	4I, 2R	2A, 1I, 3R	2I, 4R	4I, 2R
12:55	6A	6A	1I, 5R	2R, 4D	4R, 2D	3R, 3D	1I, 5D	6A	5A, 1I	4I, 2R	3I, 3R	3I, 3R	4R, 1D	6R
13:00	6A	6A	6R	1R, 5D	4R, 2D	6D	6D	6A	4A, 2I	4I, 2R	5R, 1D	3R, 3D	3R, 3D	5R, 1D
13:05	6A	5A, 1I	5R, 1D	6D	1R, 5D	—	—	6A	4A, 2I	4I, 2R	5R, 1D	3R, 3D	3R, 3D	5R, 1D
13:10	6A	2A, 4I	5R, 1D	—	1R, 5D	—	—	6A	3A, 3I	4I, 2R	5R, 1D	3R, 3D	6D	6D
13:15	6A	6I	2R, 4D	—	6D	—	—	6A	3A, 3I	4I, 2R	6D	5R, 1D	—	—
13:20	6A	3I, 3R	6D	—	—	—	—	6A	2A, 4I	2I, 4R	—	6D	—	—
13:25	6A	1I, 5R	—	—	—	—	—	6A	6I	4R, 1D	—	—	—	—
13:30	6A	1I, 5R	—	—	—	—	—	6A	1I, 5R	3R, 3D	—	—	—	—
13:35	6A	1I, 3R, 2D	—	—	—	—	—	6A	1I, 5R	3R, 3D	—	—	—	—
13:40	6A	3R, 3D	—	—	—	—	—	6A	6R	3R, 3D	—	—	—	—
13:45	6A	2R, 4D	—	—	—	—	—	6A	4R, 2D	2R, 4D	—	—	—	—
13:50	6A	2R, 4D	—	—	—	—	—	6A	4R, 2D	2R, 4D	—	—	—	—
13:55	6A	2R, 4D	—	—	—	—	—	6A	4R, 2D	1R, 5D	—	—	—	—
14:00	6A	2R, 4D	—	—	—	—	—	6A	4R, 2D	1R, 5D	—	—	—	—
14:05	6A	2R, 4D	—	—	—	—	—	6A	4R, 2D	6D	—	—	—	—
14:10	6A	1R, 5D	—	—	—	—	—	6A	3R, 3D	—	—	—	—	—
14:15	6A	1R, 5D	—	—	—	—	—	6A	3R, 3D	—	—	—	—	—
14:20	6A	6D	—	—	—	—	—	6A	2R, 4D	—	—	—	—	—
14:25	6A	—	—	—	—	—	—	6A	2R, 4D	—	—	—	—	—

-continued-

Table 9.–Page 2 of 2.

Time	Fish status <sup>a</sup>													
	No organic loading bioassay <sup>b</sup> rotenone concentration (ppb)							Organic loading bioassay <sup>c</sup> rotenone concentration (ppb)						
	0.0	12.5	25	40	50	100	200	0.0	12.5	25	40	50	100	200
14:30	6A	–	–	–	–	–	–	6A	1R, 5D	–	–	–	–	–
14:35	6A	–	–	–	–	–	–	6A	1R, 5D	–	–	–	–	–
14:40	6A	–	–	–	–	–	–	6A	6D	–	–	–	–	–

*Note:* An endash indicates no observation.

<sup>a</sup> Number of fish and status where A = no observed effect, I = impaired (erratic swimming, gulping at surface), R = rolled (rolled on side but still gilling), and D = dead.

<sup>b</sup> No lake organics added to bioassay container with 20 L Ranchero Lake water.

<sup>c</sup> One-half cup lake sediment and 2/3 cup aquatic vegetation added to each bioassay container filled with 20 L Ranchero Lake water.

<sup>d</sup> Set up refers to the bioassay container being filled with water and dosed with rotenone.

## **TREATMENT OVERVIEW**

A safety and training meeting for all ADF&G staff assisting with the project was held on 5 October 2018 at the Soldotna ADF&G office. Electronic format self-training material was provided to staff who could not attend in person. The training explained all the safety, first aid, and hazard communication required and was based on safety training information provided in (Finlayson et al. 2010). All available application staff went to the treatment area to become familiarized with the area and access points.

The TRL rotenone application began on the morning of 8 October 2018 with boat operators applying rotenone to Leisure Lake and Leisure Pond. Backpack applicators treated the Leisure Lake outlet creek including wetlands and several small ephemeral creeks that are tributaries of Leisure Pond. Applicators using an outboard-powered boat treated Leisure Lake, and applicators operating an outboard-powered canoe treated Leisure Pond. These treatments began at 1030 hours and concluded by 1530 hours.

On 9 October 2018, CC Lake, Hope Lake, and Ranchero Lake were treated with rotenone by applicators using outboard-powered boats. At Hope Lake, a boat specially equipped with a deep-water application apparatus was used. Backpack applicators applied rotenone to inundated wetlands and tributaries associated with these lakes. The rotenone application began at 1100 hours and concluded by 1600 hours.

On 10 October 2018, outboard-powered boat operators applied rotenone to Crystal Lake, operators using an outboard-powered foldable boat (Porta-Bote) treated Freds Lake. At Crystal Lake, a boat equipped with a deep-water application apparatus and another with a surface application apparatus were used. Backpack applicators treated wetlands and seeps adjacent to Freds Lake including the Freds Lake outlet creek. The rotenone applications began at 1030 and concluded by 1530.

On 11 October 2018, operators using an outboard-powered boat with a deep-water application apparatus applied rotenone to G Lake. The treatment began at 1030 hours and concluded by 1430 hours.

During the treatments, staff not applying rotenone performed tasks such as placement and monitoring of sentinel fish, placing buoy markers as visual aids to partition the lakes into treatment sections, and removing dead fish for disposal. Boat applicators, upon completing an application, would drive their boats around the lake for 30–60 minutes, creating waves to promote rotenone mixing.

In total, 92 surface acres making up 8 lakes and ponds were treated with rotenone; less than 10 acres of inundated wetlands and less than 2 miles of streams ( $<1 \text{ ft}^3/\text{s}$ ) were treated by backpack sprayers. The boat applicators were responsible for triple-rinsing empty product containers and cleaning the boats. Backpack applicators were responsible for cleaning their sprayers at the end of each day.

### **Product Applied**

Boat applicators applied 317.53 gallons of CFT Legumine to lakes in TRL. Backpack applicators applied 1.3 gallons of CFT Legumine while treating adjacent wetlands and tributary streams. A summary of the amount of CFT Legumine applied to each TRL waterbody by lake section and depth stratum is provided (Table 2).

## TREATMENT EVALUATION

The effectiveness of the rotenone treatments to eradicate each lake's northern pike or muskellunge population was assessed individually using multiple lines of evidence that included the following: (1) the observed fate of caged sentinel fish, (2) monitored rotenone concentrations, and (3) posttreatment gillnet surveys.

### Sentinel Fish

At each TRL waterbody treated with rotenone, multiple cages of sentinel fish were placed at various depths and locations to assess the efficacy of each treatment in real time. Sentinel fish were also placed in the Freds Lake outlet stream in 2 locations. In every instance, the sentinel fish died within 24 hours of the application. In most cases, the sentinel fish were dead or near death at the completion of each waterbody's treatment.

Sentinel fish were also used to assess rotenone toxicity in late November of 2018 at CC Lake, Freds Lake, G Lake, Leisure Pond, and Ranchero Lake. This was done after laboratory testing of water samples collected in early November indicated the rotenone concentration in all lakes had nearly deactivated.

In each lake where sentinel fish were deployed during November, 2 cages were placed in different lake locations and depths, and each cage held 3 juvenile coho salmon. All the sentinel fish died within 24 hours of exposure except for those in 1 cage at Ranchero Lake wherein 2 fish were severely impaired and 1 dead after 24 hours of exposure (Table 10). Also, 1 of 2 cages placed in CC Lake was lost before an observation could be made.

Table 10.—Summary of Tote Road Lakes (TRL) posttreatment sentinel fish observations, 19–30 November 2018.

Lake	Sentinel cage number	Num. of fish in cage	Cage depth (ft)	Set date/time	Check date/time	Check observation
CC Lake	1	3	1.5	29 Nov 2018/12:10	30 Nov 2018/10:45	All dead
	2	3	5.0	29 Nov 2018/12:25	30 Nov 2018/10:50	Cage lost, no observation
Freds Lake	1	3	2.0	19 Nov 2018/13:30	20 Nov 2018/12:15	All dead
	2	3	3.5	19 Nov 2018/13:40	20 Nov 2018/12:20	All dead
G Lake	1	3	1.0	29 Nov 2018/16:00	30 Nov 2018/12:10	All dead
	2	3	10.0	29 Nov 2018/16:00	31 Nov 2018/12:10	All dead
Leisure Pond	1	3	4.0	29 Nov 2018/13:10	30 Nov 2018/13:10	All dead
	2	3	4.0	29 Nov 2018/13:15	30 Nov 2018/13:10	All dead
Ranchero Lake	1	3	5.0	29 Nov 2018/11:15	30 Nov 2018/11:40	All dead
	2	3	7.0	29 Nov 2018/11:25	30 Nov 2018/10:50	1 dead, 2 severely impaired

### Rotenone and Rotenolone Monitoring

At all 8 lakes in the TRL area, including a representative private well near each, water samples were collected just before and periodically following their rotenone treatments until the rotenone was verified to have fully degraded. An exception was at Leisure Pond, where no water wells were present, so no well water samples were collected. At some lakes, 2 or more lake water samples

were collected during a single sampling event. Multiple samples collected from a lake allowed for improved assessment of how well the rotenone had mixed.

No rotenone or rotenolone was detected in any pretreatment water sample (3 October 2018). Average peak rotenone concentrations, collected 1 day after treatment (9–12 October 2018), ranged from a high of 34.3 ppb at Leisure Lake to a low of 11.3 ppb at Freds Lake (Figure 21; calculated from Table 11). The persistence behavior of rotenone at all lakes was similar, and samples collected on 10 December 2018 indicated all rotenone had fully deactivated within the TRL. At CC, Freds, and Ranchero Lakes, all samples collected on 7 November 2018 were <5.0 ppb rotenone, indicating rotenone deactivation had nearly occurred at those lakes about 5 weeks after treatment.

No pretreatment or 1-day posttreatment samples were collected from any TRL stream, but samples collected from the Leisure Pond and Freds Lake outlet streams on 7 November 2018 had rotenone concentrations of 7.0 ppb and 2.0 ppb, respectively. No rotenone or rotenolone were detected in any well water sample.

Excluding wells, peak rotenolone concentrations from individual lake samples collected 1 day after treatment (9–12 October 2018) ranged from a high of 27.2 ppb from a shallow-water sample collected at Leisure Lake on 9 October 2018 to a low of 6.4 ppb from a deep-water sample collected at Crystal Lake on 11 October 2018 (Table 12). Rotenolone persisted longer than rotenone at all lakes and creeks, with trace rotenolone ( $\leq 6.7$  ppb) still present at Leisure, Hope, and G Lakes in the last water samples collected on 8 May 2019. Rotenolone is generally regarded as about one-tenth as toxic as rotenone, and there are no regulatory thresholds or monitoring requirements for this metabolite of rotenone.

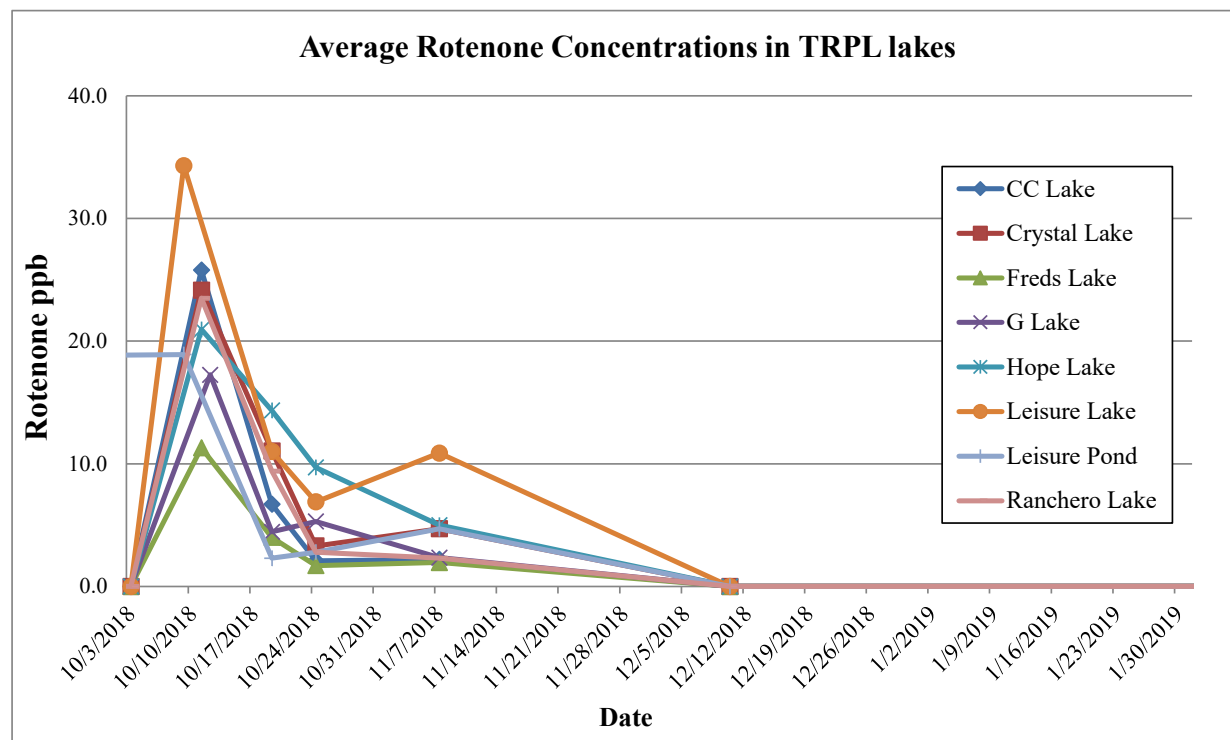


Figure 21.—Average rotenone concentrations in Tote Road Lakes (TRL) waters, October 2018–May 2019.



Table 11.—Rotenone concentrations in parts per billion (ppb) in Tote Road Lakes (TRL) waterbodies and private groundwater wells, 3 October 2018–8 May 2019.

Sample site	Sample collection date											
	2018										2019	
	3 Oct	9 Oct	10 Oct	11 Oct	12 Oct	19 Oct	24 Oct	7 Nov	10 Dec		4 Mar	8 May
Leisure Lake - near surface #1	0.0	40.8	—	—	—	21.6	6.9	12.6	0.0		0.0	0.0
Leisure Lake - near surface #2	—	—	—	—	—	—	—	14.9	0.0		—	—
Leisure Lake - deep #1	—	27.8	—	—	—	0.4	—	10.6	0.0		0.0	0.0
Leisure Lake - deep #2	—	—	—	—	—	—	—	5.4	0.0		—	—
Leisure Pond - near surface	0.0	18.9	—	—	—	2.3	2.8	4.8	0.0		0.0	0.0
Leisure Pond - deep	—	—	—	—	—	—	—	4.6	0.0		—	—
Leisure Pond outlet Cr.	—	—	—	—	—	—	3.4	7.0	0.0		0.0	—
Leisure Lake well-Bergstrom's	—	—	—	—	—	—	—	0.0	—		—	—
Leisure Lake well-Harper's	0.0	0.0	—	—	—	0.0	0.0	0.0	0.0		0.0	—
Hope Lake - near surface #1	0.0	—	18.2	—	—	13.6	8.9	5.8	0.0		0.0	0.0
Hope Lake - near surface #2	—	—	—	—	—	—	—	5.7	0.0		—	—
Hope Lake - deep #1	—	—	23.7	—	—	15.1	10.5	3.9	0.0		0.0	0.0
Hope Lake - deep #2	—	—	—	—	—	—	—	4.6	0.0		—	—
Ranchero Lake - near surface	0.0	—	23.5	—	—	9.4	2.8	2.0	0.0		0.0	0.0
Ranchero Lake - deep	—	—	—	—	—	—	—	2.6	0.0		—	—
CC Lake - near surface	0.0	—	25.8	—	—	6.7	2.1	2.2	0.0		0.0	0.0
CC Lake - deep	—	—	—	—	—	—	—	2.2	0.0		—	—
Hope/Ranchero Lake well – Stubblefield's	0.0	—	0.0	—	—	0.0	0.0	0.0	0.0		0.0	—
Hope Lake well - Svec	—	—	—	—	—	—	—	0.0	0.0		—	—
CC Lake well - Dolifka	0.0	—	0.0	—	—	0.0	0.0	0.0	0.0		0.0	—
Crystal Lake - near surface #1	0.0	—	—	40.3	—	20.2	4.1	2.4	0.0		0.0	0.0
Crystal Lake - near surface #2	—	—	—	—	—	—	—	7.0	0.0		—	—
Crystal Lake - deep #1	—	—	—	8.0	—	1.9	2.5	—	0.0		0.0	0.0
Crystal Lake - deep #2	—	—	—	—	—	—	—	—	0.0		—	—
Freds Lake- near surface #1	0.0	—	—	11.3	—	4.0	1.7	2.1	0.0		0.0	0.0
Freds Lake- near surface #2	—	—	—	—	—	—	—	1.8	0.0		—	—
Freds Lake outlet CK	—	—	—	—	—	—	1.3	2.0	0.0		0.0	0.0
Crystal Lake well-Anthony's	0.0	—	—	0.0	—	0.0	0.0	0.0	0.0		—	—
Crystal Lake well-Larson	—	—	—	—	—	—	—	0.0	0.0		—	—
Freds Lake well-Pipkin's	0.0	—	—	0.0	—	0.0	0.0	0.0	0.0		0.0	—

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Table 11.–Page 2 of 2.

Sample site	Sample collection date										
	2018									2019	
	3 Oct	9 Oct	10 Oct	11 Oct	12 Oct	19 Oct	24 Oct	7 Nov	10 Dec	4 Mar	8 May
G Lake - near surface #1	0.0	—	—	—	27.8	0.0	4.8	2.1	0.0	0.0	0.0
G Lake - near surface #2	—	—	—	—	—	—	—	2.6	0.0	—	—
G Lake - deep #1	—	—	—	—	6.7	8.9	5.8	—	0.0	0.0	0.0
G Lake - deep #2	—	—	—	—	—	—	—	—	0.0	—	—
G Well-Metzger's	0.0	—	—	—	0.0	0.0	0.0	0.0	0.0	0.0	—

*Note:* An en dash means no observation.

Table 12.—Rotenolone concentrations in parts per billion (ppb) in Tote Road Lakes (TRL) waterbodies and private groundwater wells, 3 October 2018 through 8 May 2019.

Sample Site	Sample collection date										
	2018								2019		
	3 Oct	9 Oct	11 Oct	11 Oct	12 Oct	19 Oct	24 Oct	7 Nov	10 Dec	4 Mar	8 May
Leisure Lake - near surface #1	0.0	27.2	—	—	—	18.3	13.7	10.9	4.8	7.0	3.7
Leisure Lake - near surface #2	—	—	—	—	—	—	—	12.9	—	—	—
Leisure Lake - deep #1	—	15.5	—	—	—	9.7	—	10.4	7.8	7.5	6.0
Leisure Lake - deep #2	—	—	—	—	—	—	—	9.0	—	—	—
Leisure Pond - near surface	0.0	18.4	—	—	—	5.4	7.5	7.1	2.1	2.3	0.0
Leisure Pond - deep	—	—	—	—	—	—	—	5.9	—	—	—
Leisure Pond outlet Cr.	—	—	—	—	—	—	7.7	7.1	2.1	2.9	0.0
Leisure Lake well-Bergstrom’s	—	—	—	—	—	—	—	0.0	—	—	—
Leisure Lake well-Harper’s	0.0	0.0	—	—	—	0.0	0.0	0.0	0.0	0.0	—
Hope Lake - near surface #1	0.0	—	15.1	—	—	16.9	13.5	9.1	6.9	5.8	0.0
Hope Lake - near surface #2	—	—	—	—	—	—	—	10.3	5.3	—	—
Hope Lake - deep #1	—	—	19.3	—	—	20.0	14.2	8.5	—	0.0	0.0
Hope Lake - deep #2	—	—	—	—	—	—	—	10.1	—	—	—
Ranchero Lake - near surface	0.0	—	18.3	—	—	11.9	9.3	6.5	3.1	5.4	6.7
Ranchero Lake - deep	—	—	—	—	—	—	—	5.4	—	—	—
CC Lake - near surface	0.0	—	18.6	—	—	12.7	8.6	5.7	1.9	4.3	0.0
CC Lake - deep	—	—	—	—	—	—	—	5.4	—	—	—
Hope Lake well – Stubblefield’s	0.0	—	0.0	—	—	0.0	0.0	0.0	0.0	0.0	
Hope Lake well – Svec’s	—	—	—	—	—	—	—	0.0	—	—	—
CC Lake well - Dolifka	0.0	—	0.0	—	—	0.0	0.0	0.0	0.0	0.0	—
Crystal Lake - near surface #1	—	—	—	17.4	—	15.1	11.0	2.7	3.2	3.7	0.0
Crystal Lake - near surface #2	—	—	—	—	—	—	—	7.8	—	—	—
Crystal Lake - deep #1	—	—	—	6.4	—	10.0	9.3	—	7.5	5.8	0.0
Crystal Lake - deep #2	0.0	—	—	—	—	—	—	—	—	—	—
Freds Lake- near surface #1	—	—	—	14.5	—	7.2	5.9	2.3	4.0	5.7	0.0
Freds Lake- near surface #2	—	—	—	—	—	—	—	2.0	—	—	—
Freds Lake outlet CK	0.0	—	—	—	—	—	5.0	2.2	6.9	1.9	0.0
Crystal Lake well-Anthony’s	—	—	—	0.0	—	0.0	0.0	0.0	0.0	—	—
Crystal Lake well-Larson’s	—	—	—	—	—	—	—	0.0	—	—	

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Table 12.–Page 2 of 2.

Sample Site	Sample collection date										
	2018								2019		
	3 Oct	9 Oct	11 Oct	11 Oct	12 Oct	19 Oct	24 Oct	7 Nov	10 Dec	4 Mar	8 May
Freds Lake well-Pipkin's	0.0	—	—	0.0	—	0.0	0.0	0.0	0.0	0.0	—
G Lake - near surface #1	0.0	—	—	—	13.5	6.3	10.6	2.3	1.6	4.3	4.9
G Lake - near surface #2	—	—	—	—	—	—	—	2.9	—	—	—
G Lake - deep #1	—	—	—	—	11.7	11.5	10.3	—	4.4	3.2	5.2
G Lake - deep #2	0.0	—	—	—	—	—	—	—	—	—	—
G Well-Metzger's	—	—	—	—	0.0	—	0.0	0.0	0.0	0.0	—

*Note:* An endash means no observation.

## Posttreatment Gillnet Surveys

Starting 31 October 2018 through 14 November 2018, gillnets were set in TRL waters just prior to each lake's ice-up, except Freds Lake, which was only fished in the spring of 2019 after ice-out. All nets set prior to freeze-up were fished continuously until they were removed immediately at ice-out between 16 and 19 April 2019. A total of 20 under-ice gillnets were in all lakes excluding Freds Lake, and no fish of any kind were caught (Table 13). At Freds Lake, 16 gillnets were set on 9 May 2019 and removed on 10 May 2019; no fish were caught. At all lakes, enough netting effort was applied such that the estimated probability of detecting a small surviving population of 20 northern pike was >99% (Appendix A2). The combined hours of all netting effort was 76,875, but when this effort was adjusted to account for only the duration a northern pike could be expected to remain identifiable (48 days; Dunker et al. 2016) prior to removal at ice-out, the adjusted effort represents 23,584 hours.

Table 13.—Tote Road Lakes (TRL) posttreatment gillnetting results for evaluating the treatment success.

Lake	Surface acres	Number of nets <sup>a</sup>	Set date (m/d/y)	Pull date (m/d/y)	Catch	Hours of netting effort	Adjusted hours of netting effort <sup>b</sup>	Probability of pike detection <sup>c</sup>
CC Lake	4	2	11/14/2018	04/18/2019	No fish	7,438	2,304	> 99%
Crystal Lake	17	4	11/10/2018	04/17/2019	No fish	15,117	4,608	> 99%
Freds Lake	6	16	05/09/2019	05/10/2019	No fish	544	544	> 99%
G Lake	17	4	11/18/2018	04/19/2019	No fish	14,475	4,608	> 99%
Hope Lake	27	4	11/04/2018	04/16/2019	No fish	15,636	4,608	> 99%
Leisure Lake	11	2	11/16/2018	04/17/2019	No fish	7,267	2,304	> 99%
Leisure Pond	2	2	10/31/2018	04/21/2019	No fish	8,245	2,304	> 99%
Ranchero Lake	8	2	10/31/2018	04/19/2019	No fish	8,153	2,304	> 99%
Total	92	36	—	—	No fish	76,875	23,584	—

<sup>a</sup> Gillnets were made with floating hanging lines and bottom lead lines and all were 120 ft in length, 6 ft deep and composed of 6 different monofilament mesh panels in the following sizes: 0.75 in, 1.00 in, 1.25 in, 1.50 in, 1.75 in, and 2.00 in.

<sup>b</sup> Estimated duration of under-ice netting effort wherein it is unlikely a netted northern pike would decompose and be undetectable (48 days or 1,152 hours) per information described in Dunker et al. (2016).

<sup>c</sup> Estimated probability of detecting a population of 4 surviving northern pike based on the adjusted hours of netting effort (Appendix A2).

## BIOLOGICAL MONITORING

### Fish Surveys

Native fish surveys for the TRL will be done to assess species composition, CPUE, and size (FL) under a different project that will be conducted during 2021 (Massengill et al. 2020). Results will be included in an upcoming Fisheries Management Report (FMR) for the Northern Kenai Peninsula Management Area (NKPMA).

### Invertebrate Surveys

ADF&G conducted 1 invertebrate survey at Hope Lake during 2018 and 2 surveys during 2019 (Table 14). The count of pretreatment invertebrate taxa identified to the level of order or family was 16 and similarly, the peak posttreatment invertebrate taxa count was 14 (Table 14).

More comprehensive invertebrate surveys were conducted at all 8 treated lakes by researchers from the University of Quebec (Montreal) in June of 2018 and 2019. These results included counts

of detected taxa (Appendix F1). The posttreatment taxa richness indicated an increase over pretreatment richness, although the number of sample sites at each lake doubled from ( $N = 4$ ) before treatment to ( $N = 8$ ) after treatment.

Table 14.–Hope Lake invertebrate taxa detected before and after treatment by ADF&G, 2018 and 2019.

Hope Lake invertebrate taxon	Pretreatment	Posttreatment	
	30 August 2018	19 August 2019	8 October 2019
Anisoptera (dragonflies)	Y	Y	Y
Amphipoda	Y	Y	Y
Annelida (segmented worms)	Y	–	–
Araneae (spiders/mites)	Y	Y	–
Chironomidea (non-biting midges)	Y	Y	Y
Cladocera (water fleas)	Y	Y	Y
Coleoptera (beetles)	–	Y	Y
Copepoda (Cyclopoid)	Y	Y	Y
Corixidae (water boatmen)	Y	Y	Y
Dipteran sp. (flies)	Y	–	–
Ditiscidae (predaceous diving beetle, whirligig)	Y	–	–
Gyrinidae	Y	–	–
Gastropoda (snails)	Y	Y	–
Kellicota (rotifer)	Y	–	–
Pelecypoda (molluscs)	Y	Y	–
Mollusca	Y	Y	–
Rotifera (Asplancha)	–	Y	–
Trichoptera (caddis flies)	Y	Y	Y
Zygoptera (damselflies)	–	Y	Y
Total taxa detected	16	14	9

## NATIVE FISH RESTORATION

Beginning in 2019, wild rainbow trout and coho salmon fingerlings were relocated from Soldotna Creek to TRL to establish a replacement sport fishery. Threespine stickleback introductions were completed in 2019 by university researchers in coordination with ADF&G. Releases resumed in 2020 with rainbow trout and coho salmon fingerlings collected from Soldotna Creek, Slikok Creek, and Beaver Creek. ADF&G plans to continue similar annual stocking of wild rainbow trout and coho salmon fingerlings until at least 2023.

The number of rainbow trout and coho salmon released into the TRL in 2019 was 1,844 and 7,528, respectively, totaling 9,372 salmonids (Table 15). The overall density of salmonid stocking in 2019 was 102 salmonids/surface acre. The number of threespine stickleback released by university researchers into the TRL during 2019 was 8,740.

In 2020, 2,032 rainbow trout and 7,850 coho salmon fingerlings were released into the TRL, totaling 9,882 salmonids, producing an overall stocking density of 108 salmonids/surface acre that year. No threespine stickleback were released in 2020. The annual goal of a releasing wild salmonids at a density of at least 50–100 fish/acre was met in both 2019 and 2020. This goal was listed in the project’s treatment plan.<sup>4</sup>

<sup>4</sup> Unpublished document titled “Treatment Plan: Tote Road Pike Lakes Restoration: Northern Pike Eradication” authored by Rob Massengill, ADF&G fisheries biologist, archived in the Soldotna ADF&G office.

Table 15.—Releases of wild fish into the Tote Road Lakes (TRL), 2018 and 2019.

Year	Release location	Surface acres	Salmonid releases			Salmonids/acre <sup>a</sup>	Threespine stickleback <sup>b</sup>
			Coho salmon	Rainbow trout	All salmonids		
2019	CC Lake	4	433	97	530	120	797
	Crystal Lake	17	1,389	331	1,720	103	1,494
	Freds Lake	6	552	58	610	100	419
	G Lake	17	1,380	358	1,738	100	1,653
	Hope Lake	27	2,180	591	2,771	103	1,654
	Leisure Lake	11	868	219	1,087	98	1,505
	Leisure Pond	2	120	33	153	90	412
	Ranchero Lake	8	606	157	763	99	806
	Total	92	7,528	1,844	9,372	102	8,740
2020	CC Lake	4	345	116	461	105	0
	Crystal Lake	17	1,399	356	1,755	105	0
	Freds Lake	6	476	133	609	100	0
	G Lake	17	1,429	377	1,806	104	0
	Hope Lake	27	2,018	595	2,613	97	0
	Leisure Lake	11	881	242	1,123	101	0
	Leisure Pond	2	117	33	150	88	0
	Ranchero Lake	8	1,185	180	1,365	177	0
	Total	92	7,850	2,032	9,882	108	0
Grand total			15,378	3,876	19,254	210	8,740

<sup>a</sup> Salmonids/acre equals the total number of rainbow trout and coho salmon released then divided by the lake's surface acreage.

<sup>b</sup> The threespine stickleback release data were provided by Dr. Andrew Hendry of McGill University who was part of a team of researchers that collected the sticklebacks from various southcentral Alaska populations and released them in the TRL with the intent to study their adaptive evolutionary responses and influences on lake ecology.

## DISCUSSION

### TREATMENT SUCCESS EVALUATION

Multiple lines of evidence were used to evaluate the success of eradicating northern pike and muskellunge from the TRL. These evaluation methods included gillnet surveys, sentinel fish fates, and the monitoring of rotenone concentration and persistence. Collectively, the weight of evidence suggests this project was successful at eradicating these species. Anecdotally, no reports have been received by ADF&G of any nonnative fish captured in the TRL since 2018, nor have any catches of nonnative species, and specifically northern pike, been reported to the ADF&G Statewide Harvest Survey from any Kenai Peninsula waters following the 2018 rotenone treatment.<sup>5</sup>

### ROTENONE PERSISTENCE

Between 2008 and 2018, ADF&G successfully conducted fall rotenone treatments at 11 lakes and ponds in southcentral Alaska (Massengill 2014a; Massengill 2014b; Massengill 2017b; Massengill 2022; Kristine Dunker, ADF&G Fisheries Biologist, Anchorage, personal communication). Rotenone persistence amongst these projects ranged from a minimum of 136 days at Stormy Lake (Massengill 2017b) to a maximum of 247 days at Scout Lake

<sup>5</sup> Alaska Sport Fishing Survey database [Internet]. 1996–present. Anchorage, AK: Alaska Department of Fish and Game, Division of Sport Fish (cited 2022). Available from: <http://www.adfg.alaska.gov/sf/sportfishingsurvey/>.

(Massengill 2014a). Rotenone fully deactivated in the TRL within 60 days, far faster than previously observed. It is remarkable that the persistence of rotenone in all waters treated in the TRL behaved so similarly despite significant differences in lake depth.

Environmental factors that affect the persistence of rotenone include light, heat, dissolved solids, pH, alkalinity, and biotic mechanisms (Finlayson et al. 2000; Ling 2003; Finlayson et al. 2010; Finlayson et al. 2014; Couture et al. 2020; Redman et al. 2021). A factor that may have contributed to the shorter persistence of rotenone in the TRL was the relatively warmer water temperatures. For example, Hope Lake, the largest of the TRL lakes treated with rotenone, had an average water temperature of 10.9°C on 9 October 2018. Comparatively, Stormy Lake was 5.9°C on a similar date in 2012. Gilderhus et al. (1986, 1988) examined the fate of rotenone in shallow ponds at differing temperatures and found that rotenone loss was 10 times faster at 23°C than at 1°C, which exemplifies how increasing temperature can speed degradation.

## **NATIVE FISH RESTORATION**

The long-term goals for native fish establishment in the TRL includes reestablishing self-sustaining populations of threespine stickleback and creating a new sport fishery for salmonids. Ideally, this sport fishery would become self-sustaining for rainbow trout because sustainable populations of this species are found in similar habitats on the Kenai Peninsula. A nearby drainage with similar habitat as the TRL (Bottleneck Lake system in the Kasilof River drainage) supports a wild, self-sustaining rainbow trout population. However, most of the TRL waterbodies are relatively shallow and summertime water temperatures approach the upper long-term thermal tolerances for most other resident salmonid species (e.g., Dolly Varden and Arctic char), and available spawning habitat is scarce and of poor quality.

Threespine stickleback are the only fish species native to the TRL. Other species such as rainbow trout and Chinook salmon have been reported to be caught there by area residents, mostly during the 1970s and 1980s. These fish probably resulted from unpermitted introductions. Area residents reported catching both species at Hope Lake and rainbow trout from Crystal Lake until introduced northern pike dominated these lakes in the late 1980s. Residents also reported observing rainbow trout spawning in the Hope Lake outlet stream decades ago. It is unclear if that spawning was successful at producing offspring. No interviewed residents recalled ever observing juvenile rainbow trout in the TRL. Based on site observations of the TRL outlet streams, very little spawning habitat (i.e., with well oxygenated flowing water and gravel substrate) is available. These field observations indicate that the outlet streams of Hope Lake and Freds Lake might provide limited spawning habitat in very short reaches during optimal flow conditions. ADF&G will monitor these creeks into the future to see if the newly introduced rainbow trout attempt to spawn in them. ADF&G will also minnow trap in the TRL during midsummer, when young-of-year (YOY) rainbow trout are large enough to recruit to minnow traps based on observations of YOY rainbow trout catches in late July in the nearby Soldotna and Slikok Creeks (R. Massengill, ADF&G Division of Sport Fish Biologist, personal observation).

The first releases of rainbow trout into the TRL occurred in 2019, and most of these fish were about 1 year old. Rainbow trout can become sexually mature at age 2 or 3 years, so attempts at spawning in the TRL may have occurred as early as the spring of 2020, but no spawning was observed then by ADF&G (information on rainbow trout maturity was accessed online on 8 December 2020 at <http://www.adfg.alaska.gov/index.cfm?adfg=steelhead.main>).



Most TRL outlet streams discharge less than 1 ft<sup>3</sup>/s year-round, and gravel substrate is nearly absent from most streams. Improving the rainbow trout spawning habitat in select TRL outlet creeks may be considered in the future. Such improvements could include adding gravel substrate and creating more compressed stream channels to improve flow. Any successful spawning habitat improvement efforts would first require public scoping and engineering plans. One TRL lakeside resident suggested that local residents may be willing to help fund spawning habitat improvements.

All the TRL waterbodies except for G Lake are linked by small, often ephemeral, streams providing potential seasonal fish passage. Hope Lake and Ranchero Lake are connected by a deep channel that provides easy year-round inter-lake fish passage (Figure 22).



Figure 22.—Canal linking Hope Lake to Ranchero Lake.

The stream linking Ranchero Lake to Crystal Lake, where historically, rainbow trout were observed spawning in the 1970s and 1980s, appears to offer the best potential spawning habitat despite 2 perched culverts and a manmade earthen berm impounding the stream (Figure 23).



Figure 23.—Ranchero Lake outlet creek where anecdotal reports suggest rainbow trout attempted to spawn in the 1970s and 1980s.

Crystal Lake and Freds Lake are connected by a stream that also appears capable of providing fish movement for much of the year. At other TRL lakes, their outlet creeks pose severe challenges to fish movement, either due to low discharge, or because the stream is perched by human alterations (e.g., culverts, earthen berms) such as those that exist between CC and Hope Lakes and between Ranchero and Crystal Lakes. At Leisure Lake, the outlet leading to Leisure Pond is perched by an old beaver dam, which impounds Leisure Lake several feet above static height.

At G lake, due to its poor connectivity to any other waters and lack of suitable rainbow trout spawning habitat, some level of salmonid stocking will clearly be needed to continue a salmonid fishery beyond 2023. In all the TRL waters, reintroduced threespine stickleback are expected to be self-sustaining because they are generally regarded as a hardy species and tolerant of a wide range of habitat conditions (Östlund-Nilsson et al. 2006).

Eliminating impediments to fish passage in the TRL might improve rainbow trout access to the limited spawning habitat found there, and juvenile rainbow trout would be able to move among the lakes. Natural dispersal of rainbow trout could be particularly beneficial for those lakes devoid of spawning habitat in their outlet streams. However, the removal of fish passage obstacles, like perched culverts and beaver dams, could lower the height of some lakes, which could cause concern to lakeside residents.

Other options to consider for maintaining a salmonid sport fishery in the TRL beyond 2023 include the following:

- 1) continuing the release of wild salmonids
- 2) stocking some lakes with classroom-reared coho salmon through the local ADF&G Salmon in the Classroom program
- 3) stocking some lakes with hatchery-produced fish

These options were all shared with the public during project scoping meetings in 2017, particularly attended by TRL lakeside residents, who were generally not supportive of stocking hatchery-produced fish into the TRL due to the concerns over public access requirements to these lakes.

The university researchers involved with the TRL threespine stickleback releases reported that their 2020 minnow trapping surveys conducted at all TRL lakes found stickleback present in all TRL lakes except G Lake. Further investigations will reveal if more threespine stickleback releases at G Lake are warranted. By the fall 2019, ADF&G began receiving occasional reports from TRL anglers about catching rainbow trout and coho salmon, and that threespine stickleback were visually observed in most TRL lakes.

## **RECENT NONNATIVE FISH DISCOVERIES**

The discovery of muskellunge in G Lake underscores the concern that some people will go to great effort to illegally introduce fish. The nearest location to G Lake where muskellunge naturally exist is in Manitoba, Canada, about 1,500 miles distant. There is no conceivable way the G Lake muskellunge could have arrived in Soldotna naturally. The genetic analysis of the G Lake muskellunge indicates these fish probably originated from northern Wisconsin. Ages of the recovered muskellunge were determined by cleithra annulus, and the oldest muskellunge was about 11 years old. Assuming the oldest fish captured was an original founder and introduced in its first year of life, the muskellunge introduction may have occurred around 2006 at the earliest. The ages of captured muskellunge ranged from 11 to 2–3 years. This age variation could be the result of several scenarios:

- 1) a single introduction sometime during 2015–2017 involving a wide range of muskellunge ages and sizes
- 2) multiple illegal introductions occurring over several years
- 3) a single release event resulting in a reproducing population

A lakeside resident of G Lake reported seeing large “pike-like” fish in G Lake for at least several years prior to their discovery by ADF&G in 2017. Multiple introductions of muskellunge to G Lake across years is plausible but greatly increases the risk and transport challenges to the perpetrator. Investigations by state and federal law enforcement led to a suspect being questioned, although no charges resulted.

In May 2017, an angler reported catching a northern pike at Warfle Lake in Kasilof, located about 10 miles south of the TRL. Subsequent gillnet and minnow trap surveys by ADF&G confirmed the presence of northern pike and the absence of threespine stickleback, a species some lakeside residents reported as once being common to the lake. Six northern pike were collected by ADF&G gillnetting efforts in May 2017, and another 7 angler-caught northern pike were provided to ADF&G. An unknown additional number of northern pike were harvested by anglers. All fish examined were emaciated (Figure 24), 9 fish were identified for sex, and all of them were female. An area resident reported that people first started fishing at Warfle Lake around 2007, presumably

coinciding near the time of the northern pike introduction. One person reported to ADF&G the name of the person they suspected introduced the northern pike; this report was forwarded to law enforcement.

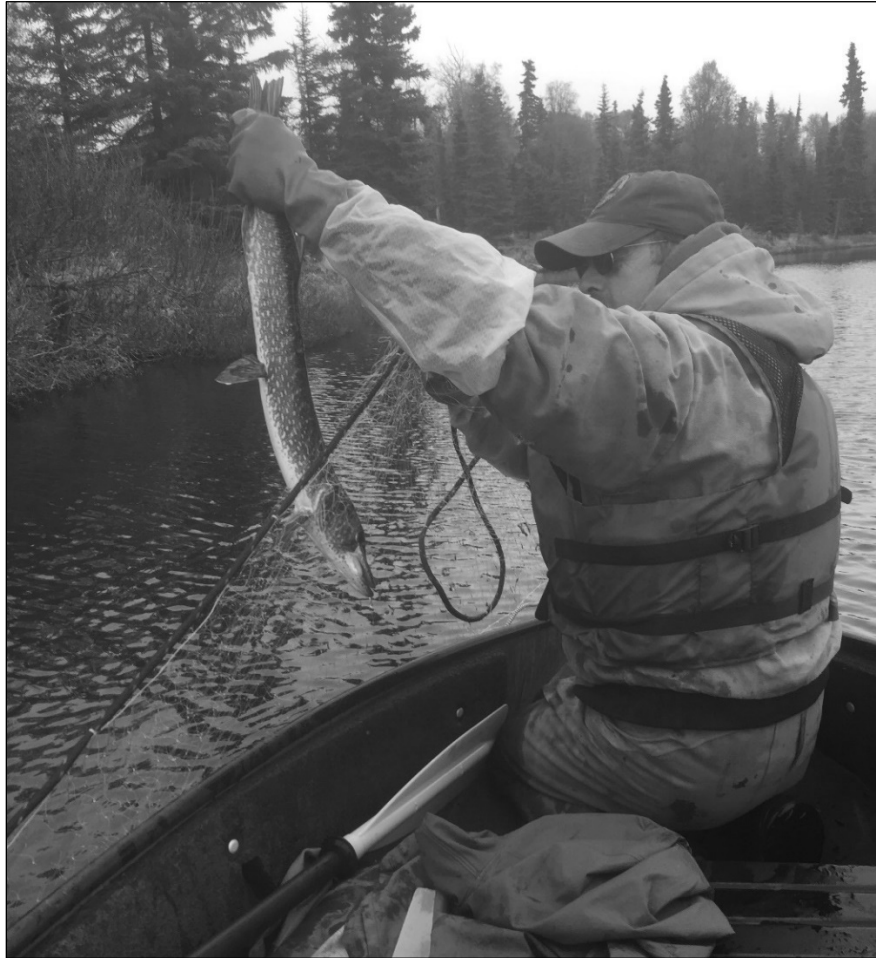


Figure 24.—Emaciated northern pike captured at Warfle Lake, 2017.

ADF&G decided to eradicate the Warfle Lake northern pike population by first trying mechanical removal using gillnets. Eradication by mechanical removal was deemed practical because the lake is very small (about 7 surface acres), the abundance of northern pike appeared very low based on gillnet CPUE, and no juvenile northern pike had been captured, suggesting reproduction might not be occurring. During October 2017, over 4,300 hours of gillnetting effort was expended in Warfle Lake and no fish were caught. By estimating the northern pike detection probability from this amount of netting as described in Appendix A2 (>90%), ADF&G concluded the northern pike population was likely eradicated by the previous gillnetting and angling done during May of that year. A 2020 gillnet survey at Warfle Lake also verified this lack of northern pike. In 2018, university researchers reintroduced threespine stickleback to Warfle Lake.

Illegal fish introductions to the Kenai Peninsula are not uncommon as evidenced by the recent discoveries of esocids and other nonnative fish species detected since 2017. These introductions include:

- 1) fathead minnows (*Pimephales promelas*) at a 1-acre pond in Kenai that were removed via rotenone by ADF&G in 2019
- 2) a nonnative strain of rainbow trout, illegally released in a small landlocked lake in Kasilof where ongoing ADF&G-led gillnet efforts to remove them are occurring
- 3) unpermitted nonnative blackfish, discovered abandoned in a container left at the Kenai Airport in January 2020

Nonnative fish discoveries in other parts of southcentral Alaska since 2017 include largemouth bass (*Micropterus salmoides*) and goldfish (*Carassius auratus*) in Anchorage at Sand Lake and Cuddy Pond, respectively. New reports of northern pike occur regularly in closed lakes in the Matanuska-Susitna Valley north of Anchorage (personal communication, Parker Bradley, Fisheries Biologist, ADF&G, Palmer, Alaska)

Unfortunately, northern pike were confirmed present in 2019 in the Miller Creek drainage located near the northern tip of the Kenai Peninsula just south of Point Possession. Currently, there are cooperative efforts between ADF&G and the USFWS to plan and implement a response action to address this concern, which will be in a future ADF&G Special Publication series report.

These nonnative fish introductions demonstrate the need for continued public education and outreach about the environmental and legal consequences of illegal fish introductions. Enforcement of existing laws regarding the transport and release of live fish, and the conviction of offenders, should help dissuade those from considering illegal releases in the future. The first conviction in Alaska of someone illegally transporting and releasing fish occurred in 2020 when a strain of nonnative hatchery-reared rainbow trout was imported to Alaska and released into a lake in Kasilof. Ongoing monitoring efforts to detect invasive fish on the Kenai Peninsula should continue because early detection and rapid response are invariably the most cost-effective means to address invasion threats beyond preventing their occurrence.

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**APPENDIX A: CALCULATING THE PROBABILITY OF  
FAILING TO DETECT NORTHERN PIKE WITH VARIOUS  
SAMPLING EFFORTS**

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Appendix A1.—Calculating the probability of failing to detect northern pike with eDNA sampling efforts.

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To develop an eDNA sampling effort that is sufficiently robust to detect northern pike populations with low abundance, the estimated mean detection probabilities of northern pike eDNA reported in Dunker et al. (2016) were utilized. The detection probabilities were estimated from results using replicate 1-liter samples collected at 1, 10, and 40 meters from a single, caged, live northern pike and were estimated to be 0.89, 0.57, and 0.27, respectively.

The following calculations will be used to estimate how many eDNA samples are needed to detect a small northern pike population ( $N = 20$ ) with a desired probability of detection provided the lake acreage is known and no gillnet sampling occurs. Calculations will be based on 3 assumptions: (1) fish are randomly distributed throughout the sampling area; (2) there are no false detections; and (3) the probability of detection beyond 40 m is zero, because no estimates are available for this range.

To account for differences in the probability of detection due to the distance between a possible northern pike and the sample site, a 40-meter circle around each sample site will be divided into 3 distinct regions centered around the sample site. These regions will be the circular area less than 1 meter from the center (the sample site) and the donut-shaped areas between 1 and 10 meters from the center and between 10 and 40 meters from the center, which will be labeled regions 1, 2, and 3 respectively. Because Dunker et al. (2016) estimated the probability of detection at 1, 10, and 40 meters, their estimates will be used as conservative proxies for the probability of detection over the entire respective regions. If  $P$  represents the probability of detecting a northern pike,  $D$  represents the event a northern pike is detected, and  $R_i$  represents the event that a single northern pike is present in region  $i$  for  $i = 1, 2$ , or  $3$ , then by the law of total probability and the definition of conditional probabilities:

$$P(D) = P(D | R_1) \times P(R_1) + P(D | R_2) \times P(R_2) + P(D | R_3) \times P(R_3) \quad (A1)$$

Thus, the probability a northern pike is detected is equivalent to the probability a northern pike is detected given it is in a particular region times the probability it is in the region summed over all regions. Under the assumption that northern pike are randomly distributed, the probability a northern pike is present in a region is the proportion of total area represented by that region or

$$P(R_i) = \frac{\text{area of region } i}{\text{total area of lake}} \quad (A2)$$

which is computed by dividing the fixed area of each circular region by the known surface area.

Finally, assuming sample sites are identical and there are no false positives, it can be shown that the probability of detection given a northern pike is at 1 sample site is equal to the probability of detection given the pike is at 1 of  $S$  sample sites for  $S = 1, 2, \dots, n$ . Thus, the only change in the probability calculation for  $S$  sites is that the proportion of area represented by each region is now  $S \times P(R_i)$ . By another application of the law of total probability and definition of conditional probabilities:

$$P(D \text{ at } S \text{ sites}) = \frac{P(D | R_1) \times S \times P(R_1) + P(D | R_2) \times S \times P(R_2) + P(D | R_3) \times S \times P(R_3)}{S \times P(D)} = \quad (A3)$$

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Because the  $N$  northern pike are assumed randomly distributed (which is a conservative assumption because samples are taken in the best northern pike habitat), the number of northern pike that are assumed successfully detected follows a  $\text{Bin}[N, S \times P(D)]$  distribution. The probability of at least 1 detection at  $S$  sites is  $1 - [1 - S \times P(D)]^N$ . This equation can then be set equal to the desired probability of detection and solved for  $S$ . Table A1 displays calculated eDNA sampling requirements for a variety of desired probabilities of detection and acreages assuming a population of 20 northern pike.

Table A1.–Number of samples required to achieve the desired probability of detection for a population of 20 northern pike.

Probability of detection	Acres					
	10	25	50	75	100	200
0.50	1	3	5	8	10	19
0.75	2	5	10	14	19	38
0.80	3	6	11	17	22	44
0.85	3	7	13	19	26	51
0.90	4	8	16	23	31	61
0.95	4	10	20	30	39	78

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Appendix A2.–Calculating the probability of failing to detect northern pike with gillnetting efforts.

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To quantify the netting effort necessary to detect a remnant surviving northern pike population of at least 4 fish with an estimated probability of detection of 80%, we utilized data from past northern pike removal experiments.

Between 2005 and 2010, ADF&G conducted 12 removal events with northern pike populations on the Kenai Peninsula using similar gillnetting methods. Data collected from these events included catch  $C_{ij}$  and effort  $E_{ij}$  (in units of net-hours per surface acre) for sample  $i$  where  $i = 1, \dots, s$  and event  $j$  where  $j = 1, \dots, 12$ . Populations were assumed to be closed except for captured fish, and fishing was assumed to represent a Poisson process with a constant probability of capture for all individuals. Data were analyzed using a hierarchical version of Leslie's regression method (Seber 1982):

$$CPUE_{ij} = K_j N_j - K_j C_{ij}^* \quad (A4)$$

where

$$CPUE_{ij} = C_{ij} / E_{ij} \quad (A5)$$

$$C_{ij}^* = \sum_{k=1}^{i-1} C_{kj} \text{ for } (i \text{ in } 2, \dots, s+1) \text{ with } C_{1j}^* = 0 \quad (A6)$$

and

$N_j$  = the initial population size in experiment  $j$ , and

$K_j$  = average probability that a northern pike of any size is captured with 1 unit of effort during experiment  $j$ .

The probabilities of capture for each experiment are assumed to come from a common distribution:  $K_j \sim \text{beta}(a, b)$ .

The analysis was conducted using the RJAGS package (Plummer 2013) within R (R Core Team 2016). Noninformative priors were used for all parameters. Although Leslie's method is typically used to estimate the initial population size, our interest was in the posterior and predictive distributions of  $K$  for the purpose of estimating the probability of detecting small northern pike populations in future (new) removal experiments.

Percentiles from the predictive distribution for the value of  $K$  in a new removal experiment are listed as follows:

Percentile	Predicted $K$
5%	0.001
10%	0.003
50%	0.019
90%	0.055
95%	0.073

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The predictive distribution for a new removal experiment is shown in Figure A2.

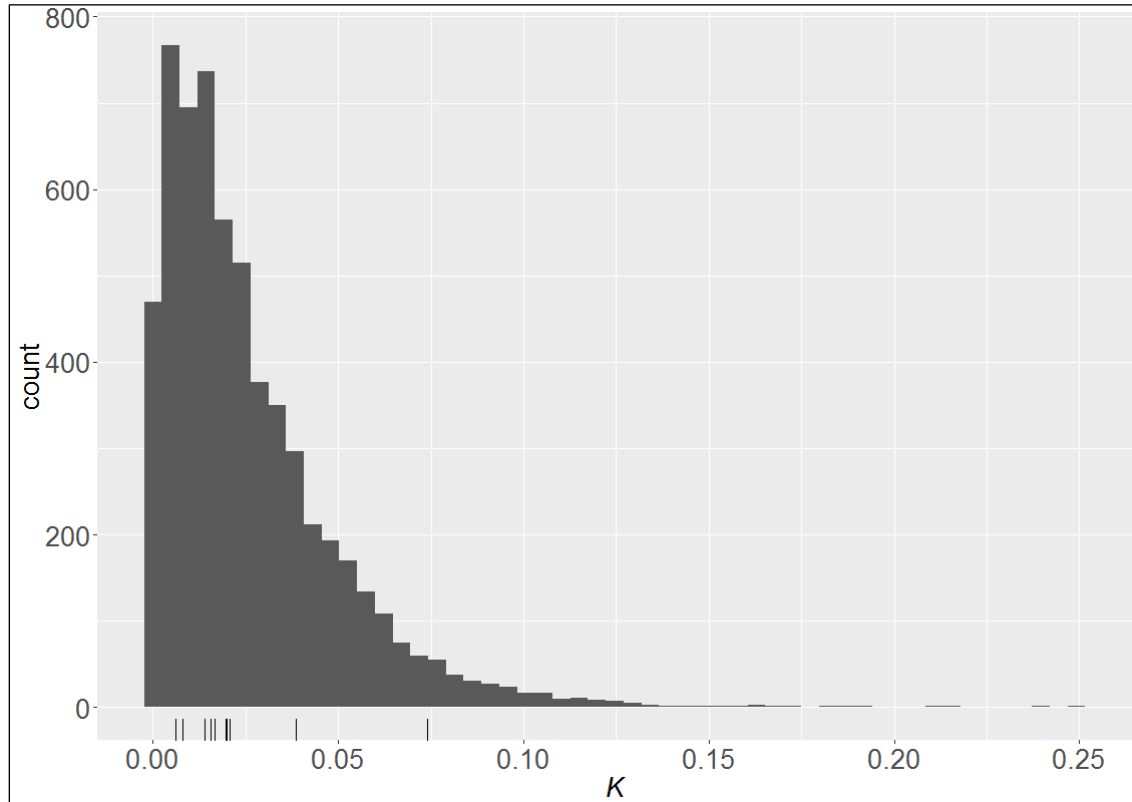


Figure A2.—Predictive distribution for  $K$ , the average probability a fish is captured in a new removal experiment with 1 unit of effort. Tick marks along the  $x$ -axis show the median values for  $K_j$ , the average probability a fish is captured with 1 unit of effort in each of the previous removal experiments.

Under the assumption that fishing represents a Poisson counting process, the probability of failing to detect a population of pike of size  $N$  as a function of net-hours per acre ( $E$ ) is

$$D_p = \exp(-KE)^N$$

We used the median value of  $K$  (0.019 from the 50th percentile listed above) to calculate the probabilities listed in Table A2, which along with effort, were used to satisfy precision criteria.

Table A2.—Probability of failing to detect a population of 4 pike with various levels of net density (nets per surface acre [sa]) and net hours given the average probability that a northern pike of any size is captured with 1 unit of effort is 0.019.

Netting hours	Net densities					
	0.1nets/sa	0.25nets/sa	0.5nets/sa	0.75nets/sa	1nets/sa	2nets/sa
24 hours	0.829	0.626	0.392	0.246	0.154	0.024
48 hours	0.688	0.392	0.154	0.06	0.024	0.001
72 hours	0.57	0.246	0.06	0.015	0.004	0
96 hours	0.473	0.154	0.024	0.004	0.001	0

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Gillnets used for northern pike surveys were identical to those used in the 12 removal events mentioned previously and the pretreatment northern pike removal activities. The gillnets were manufactured by Duluth Nets and made of single-strand monofilament mesh hung from a polypropylene floating line with the net bottom attached to 30 lb lead line. Each net was 120 ft long, 6 ft deep, with six 20 ft wide panels of different sized mesh (1 each of sequentially attached 0.5-inch, 0.625-inch, 0.75-inch, 1.0-inch, 1.5-inch, and 2.0-inch stretched mesh) all tied with #9 twine. Gillnets were deployed in vegetated littoral areas and fished continuously as practical.



## **APPENDIX B: ROTENONE PRODUCT LABEL**



# **RESTRICTED USE PESTICIDE**

Due to acute inhalation, acute oral and aquatic toxicity. For retail sale to, and use only by, Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's certification.

THE APPLICATOR IS RESPONSIBLE FOR CONFORMING TO THE LABEL. IMPORTANT GUIDANCE ON THE SAFE AND EFFECTIVE USE OF THIS PRODUCT IS PROVIDED IN THE *ROTENONE SOP MANUAL*, AVAILABLE FROM THE REGISTRANT OR THE AMERICAN FISHERIES SOCIETY AT [www.fisheries.org/units/rotenone](http://www.fisheries.org/units/rotenone)

**FOR CONTROL OF:** Fish in Lakes, Ponds, Reservoirs and Streams

# SPECIMEN LABEL

## ACTIVE INGREDIENTS:

Rotenone ..... 5% w/w  
 Cube Resins other than rotenone..... 5%  
**OTHER INGREDIENTS\***..... 90%  
**TOTAL:** ..... 100%

\*Contains Petroleum Distillates

## KEEP OUT OF REACH OF CHILDREN WARNING

See Additional First Aid, Precautionary Statements and Directions for Use including Storage and Disposal Instructions

EPA Reg.No. 89459-48

EPA Est. No. Ⓐ 44616-MO-1 Ⓑ 44616-MO-2

## PRECAUTIONARY STATEMENTS – HAZARDS TO HUMANS AND DOMESTIC ANIMALS – WARNING

May be fatal if inhaled. Do not breath the vapors or spray mists. May be fatal if swallowed. Causes moderate eye irritation. Harmful if absorbed through skin. Do not get in eyes or on skin or clothing.

FIRST AID	
Have product container or label with you when obtaining treatment advice.	
<b>If inhaled</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth, if possible.</li> <li>• Call a poison control center or doctor for further treatment advice.</li> </ul>
<b>If swallowed</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Do not give any liquid to the person.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>

<b>If in eyes</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes.</li> <li>• Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If on skin or clothing</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may contact 1-800-248-7763 for emergency medical treatment information. You may also contact the National Pesticide Telecommunication Network at 1-800-858-7378 for information including health concerns, medical emergencies or pesticide incidents.	
<b>NOTE TO PHYSICIAN:</b> Contains petroleum distillate. Vomiting may cause aspiration pneumonia. Symptoms of exposure include numbness, lethargy and incoordination. Decontamination, symptomatic and supportive treatment is recommended.	

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#### **Personal Protective Equipment (PPE)**

Some materials that are chemical resistant to this product are Barrier Laminate, Nitrile Rubber, Neoprene Rubber or Viton. If you want more options, follow the instructions for Category E on EPA chemical-resistance category selection chart.

All mixers, loaders, applicators, and other handlers (except pilots) must wear at a minimum, the following PPE: (1) coveralls, over long-sleeved shirt and long pants; (2) chemical-resistant gloves; (3) chemical-resistant footwear plus socks; (4) protective eyewear; and (5) a dust/mist respirator.

In addition, mixers, loaders, and others exposed to the concentrate, through cleaning equipment or spills must wear a chemical-resistant apron.

Exception: waterproof waders may be worn in place of coveralls, chemical-resistant apron and chemical-resistant footwear.

See Engineering Controls for additional requirements and exceptions.

#### **User Safety Requirements**

Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry. Discard clothing and other absorbent materials that have been drenched or heavily contaminated with this product's concentrate; do not reuse them. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.

#### **Engineering Controls for Mixing/Loading/Applying Liquid Formulations Packaged in Containers > 5 Gallons**

Mixers/loaders/applicators must either:

- (1) Use a closed system that meets the requirements listed in Worker Protection Standard (WPS) for dermal protection of agricultural pesticides [40 CFR 170.240(d)(4)], or
- (2) Use the Semi-Closed Probe Mixing/Loading/Applicator System described below.

Remove plug from bung of drum containing this product only when drum is sitting on the ground or on a secure level platform, with the drum pointed up. Do not pour this product from its drum.

Transfer product from the drum of the mixing tank by use of a suction hose connected to one end of the suction pump on the mixing tank and connected at the other end to a probe/dip tube. Remove the plug from the bung of the drum and insert the probe/dip tube into the bung of the drum until the foam ring/gasket fits snugly around the bung opening to minimize leakage of liquid rotenone. The probe/dip tube should be specifically sized to insure a snug fit into the bung which incorporates an anti-drip flange to remove excess liquid rotenone when the probe/dip tube is removed. In addition, the foam ring/gasket on the probe/dip tube insures a snug fit to minimize leakage of liquid rotenone. Do not handle the probe/dip tube in a manner that allows dripping or splattering of the product onto yourself or any other person. Do not touch the portion of the probe/dip tube that has been in contact with this product until the probe has been triple rinsed with water. See Rotenone SOP Manual (SOP 8) for further information on the operation of the Semi-Closed Probe system.

If the entire product is removed from the drum, then triple rinse the probe while it remains inside of the drum if possible. If not, remove the aspirator probe and triple rinse it and all parts of the aspirator in site water. If an unrinsed probe must be removed from the drum, triple rinse it and all parts of the aspirator in treated site water. The anti-drip flange must be designed to remove excess rotenone product from the probe as it is extracted from the drum. Take the following steps if the probe must

be disconnected from the suction hose before both the probe and the hose have been triple rinsed: (1) equip the probe end of the hose with a shutoff valve; (2) install a dry-brake coupling between the valve and the probe, and then close the shut off valve before disconnecting the probe. See Rotenone SOP Manual (SOP 8) for further information on unrinsed probes.

Mixers/loaders/applicators using all systems must wear PPE as required in the PPE section of this labeling for mixers/loaders. All systems must be capable of removing the pesticide from the shipping container and transferring it into mixing tanks and/or application equipment. At any disconnect point, the system must be equipped with a dry disconnect or dry-couple shutoff device to minimize drips.

#### **Transferring (Mixing/Loading) Liquid Formulations**

Mixers and loaders must transfer product from original to mixing tank or secondary container using a measuring device, inside a plastic-lined bermed area or other secondary confinement area capable of recovering spilled product. Wash plastic liner or other secondary confinement area and dispose of into treated site water. Do not handle this product in a manner that drips or splatters the product onto yourself or any other person. See Rotenone SOP Manual (SOP 10) for further guidance.

**Product Containers < 5 Gallons** – Transfer product from original container into measuring device, within secondary confinement area, by pouring or using pump or pipette-type device. See Rotenone SOP Manual (SOP 10) for further guidance.

**Product Containers > 5 Gallons** – Do not pour rotenone concentrate from containers > 5 gallons. Transfer product from original container into measuring device, within secondary confinement area, using hand or electric drum pump. See Rotenone SOP Manual (SOP 10) for further guidance.

#### **Engineering Controls for Applying Liquid Formulations**

Applications using a boom or other mechanized equipment must release this product below the water surface. Applications made with aircraft, backpack sprayer, drip can, or hand-held or hand-directed nozzle may release this product above the water surface.

#### **Engineering Controls for Aerial Applications**

Open cockpits are prohibited. Pilots must use a cockpit that has a nonporous barrier that totally surrounds the cockpit occupants and prevents contact with pesticides outside the enclosed area. Pilots in enclosed cockpits may wear a long-sleeved shirt, long pants, shoes, and socks instead of the PPE required for applicators in the PPE section of this labeling.

#### **Engineering Controls for Boat Applications**

When boat pilots or others on the application boat are located within an enclosed area that has a nonporous barrier that totally surrounds the occupants and prevents contact with pesticides outside the enclosed area; they: (1) may wear long-sleeved shirt, long pants, shoes, and socks, instead of the PPE required for applicators in the PPE section of this labeling; (2) must be provided and have immediately available in the use of an emergency when they must exit the enclosed area while the application is taking place, the PPE required for applicators of the PPE section of this labeling; (3) must take off any PPE that is worn while outside the enclosed area before reentering the enclosed area; and (4) store all used PPE in a chemical-resistant container, such as a plastic bag, to prevent contamination of the enclosed area.

#### User Safety Recommendations

Certified Applicators applying or supervising any aspect of the application of this product should attend a training program for the Rotenone SOP Manual. The American Fisheries Society offers this training; go to [www.fisheries.org/units/rotenone](http://www.fisheries.org/units/rotenone) for current schedule of training.

Users should remove clothing/PPE if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Users should remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

### ENVIRONMENTAL HAZARDS

This product is extremely toxic to fish and other aquatic organisms. Fish kills are expected at recommended rates. Consult your State Fish and Game Agency and other agencies before applying this product to public waters to determine if a permit is needed for such an application. Do not contaminate water outside of the treatment area by cleaning of equipment or disposal of equipment washwaters. Do not contaminate water outside of the treatment area, food or feed by storage or disposal. Do not discharge effluent containing this pesticide into sewage systems without notifying the sewage treatment plant authority (PTOW).

### PHYSICAL AND CHEMICAL HAZARDS

**Flammable.** Keep away from heat and open flame.

### DIRECTIONS FOR USE

#### RESTRICTED USE PESTICIDE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING, INCLUDING BOTH THE CONTAINER LABEL AND THE ROTENONE STANDARD OPERATION PROCEDURES MANUAL (SOP) available from the registrant or the American Fisheries Society at [www.fisheries.org/units/rotenone](http://www.fisheries.org/units/rotenone). THIS PRODUCT MUST BE ACCOMPANIED BY AN EPA-APPROVED ROTENONE SOP MANUAL. READ THE CONTAINER LABEL AND ROTENONE SOP MANUAL PRIOR TO USE. THE APPLICATOR IS RESPONSIBLE FOR FOLLOWING THE DIRECTIONS FOR USE CONTAINED WITHIN BOTH THE CONTAINER LABEL AND THE SOP MANUAL.

This product is registered for use by or under permit from, and after consultation with State and Federal Fish and Wildlife and/or Natural Resource Agencies.

### GENERAL INFORMATION

This product is a specially formulated product containing rotenone to be used in fisheries management for the eradication of fish from lakes, ponds, reservoirs, rivers and streams. Properly dispose of unused product. Do not use dead fish for food or feed. Do not use water treated with rotenone to irrigate crops or release within ½ mile upstream of an irrigation water intake in a standing body of water such as a lake, pond, or reservoir.

**General Application Precautions and Restrictions:** The Certified Applicator supervising the treatment must remain on site for the duration of the application. Do not allow recreational access (e.g., wading, swimming, boating, and fishing) within the treatment area while rotenone is being applied (see Placarding of Treatment Areas). In streams/rivers/lakes/reservoirs/ponds, do not apply this product in a way that will result in active rotenone concentrations > 200 parts per billion/0.2 ppm (> 4.0 ppm 5% rotenone formulation). Do not apply this product in a way that will contact workers or other persons, either

directly or through drift. Only protected handlers may be in the area during application (see Placarding Treatment Areas and Re-entering of Treatment Area). This product must not be applied to estuarine or marine environments. Where practical, users should collect and bury dead fish.

Applications using a boom or other mechanized equipment must release this product below the water surface.

Applications made with aircraft, backpack sprayer, drip can, or hand-held or hand-directed nozzle may release this product above the water surface.

Mixers/loaders of liquid rotenone product containers of 5 gallons or less should not handle more than 25 gallons of undiluted product per day.

**Re-entering the Treatment Area:** For applications that result in concentrations greater than 0.09 ppm active rotenone (when applying at a rate of > 1.8 ppm of 5% rotenone formulation), handler re-entering treated water, must wear, at a minimum, the following PPE: (1) coveralls over long-sleeved shirt and long pants; (2) chemical-resistant gloves; (3) chemical-resistant footwear plus socks; and (4) Chemical-resistant apron. Duration of PPE requirements for handlers re-entering treated water exactly corresponds to duration of placarding requirements (e.g., PPE requirements end when placards are removed; see Placarding of Treatment Areas section of this labeling). Exception: waterproof waders may be worn in place of coveralls, chemical-resistant apron and chemical-resistant footwear.

**Placarding of Treatment Areas:** The Certified Applicator in charge of the application (or someone under his/her supervision) must placard all access areas to the treatment area. Detailed instructions for placarding are presented in the Rotenone SOP Manual. Placards must be placed every 250 feet along the shoreline of the treated area OR, at public access points (e.g., trailheads, roads and trails). Placards must contain the following information: (1) DANGER/PELIGRO; (2) DO NOT ENTER WATER/NO ENTRE AGUA; Pesticide Application; (3) CTF Legumine Fish Toxicant; (4) the purpose of the application; (5) the start date and time of application; (6) end date and time of application; (7) "Recreational access (e.g., wading, swimming, boating, fishing, etc.) within the treatment area is prohibited while rotenone is being applied"; (8) "Do not swim or wade in treated water while placard is displayed"; (9) "Do not consume dead fish from treated water"; and (10) the name, address, and telephone number of the responsible agency or entity performing the application.

Signs must remain legible during the entire posting period. For lotic (flowing water) and lentic (standing water) applications of ≤ 0.09 ppm active rotenone (≤ 1.8 ppm 5% formulation), signs can be removed once application is complete. For lotic applications > 0.09 ppm active rotenone (> 1.8 ppm 5% rotenone formulation), signs can be removed 72 hours after application is complete. For lentic applications > 0.09 ppm active rotenone (> 1.8 ppm 5% rotenone formulation), signs can be removed following 24-hour bioassay demonstrating survival of bioassay sentinel fish or 14 days, whichever is less.

**Monitoring and Notification Requirements for Water Aquaculture:** For treated water bodies used for aquaculture, the Certified Applicator or designee under his/her direct supervision must prohibit the restocking of fish unless monitoring samples confirm rotenone concentrations are below the level of detection for 3 consecutive samples taken no less than 4 hours apart. Detailed guidance for monitoring levels of rotenone in water is presented in the Rotenone SOP Manual (SOP 16).

**Drinking Water:** For applications > 40 ppb or 0.04 ppm active rotenone

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(> 0.8 ppm 5 % rotenone formulation) in waters with drinking water intakes or hydrologic connections to wells, 7 to 14 days prior to application, the Certified Applicator or designee under his/her direct supervision must provide notification to the party responsible for the public water supply or individual private water users against the consumption of treated water until: (1) active rotenone < 0.04 ppm as determined by analytical chemistry, or (2) fish of the *Salmonidae* or *Centrichidae* families can survive for 24 hours, or (3) dilution with untreated water yields a calculation that active rotenone is < 0.04 ppm, or (4) distance or travel time from the application sites demonstrates that active rotenone is < 0.04 ppm. See Rotenone SOP Manual (SOP 16) for guidance on notification and bioassay and chemical analysis techniques and dilution, distance, and travel time criteria.

#### Specifications to Control Spray Drift

**RELEASE HEIGHT:** Spray must be released at the lowest height consistent with pest control and flight safety.

**BOOM LENGTH:** The boom length must not exceed 75% of the wing span or 90% of the rotor blade diameter. Orient nozzles backward with minimal downward angle into slip stream.

**SWATH ADJUSTMENT:** When applications are made with cross wind, the swath will be displaced downwind. The applicator must compensate for this displacement at the downwind edge of the application area by adjusting the path of the aircraft upwind. Leave at least one swath unsprayed at the downwind edge of the treated area.

**DROPLET SIZE:** Use low drift nozzles designed to produce larger spray droplets with fewer driftable fines. Apply as a medium or coarser spray (ASAE standard 572).

**WIND SPEED:** Do not apply when wind speeds are >12 miles per hour.

### DETERMINING TREATMENT RATE

Use this product only at locations, rates, and times authorized and approved by appropriate State and Federal Fish and Wildlife and/or Natural Resource Agencies. The actual treatment rate and rotenone concentration needed to control fish varies widely, depending on the type of water environmental factors including pH, temperature, depth, turbidity, and the target species. The tables below are a general guide for the proper rates and concentrations for complete kills of target species. The Certified Applicator must conduct bioassays using site water (or water of similar quality) and target species (or surrogate species of similar sensitivity) to refine the treatment rate with the maximum limit allowed. Detailed guidance bioassays and designing treatment for complete kills of target species are presented in the Rotenone SOP Manual (SOP 5). Rates must be within the range specified on the label.

### FOR USE IN PONDS, LAKES, AND RESERVOIRS

The tables in this booklet are a general guide for the proper rates and concentrations. This product disperses readily, laterally and vertically. For complete coverage, it is best to apply this material to water bodies that are not thermally-stratified. However, this material will eventually penetrate below the thermocline in thermally-stratified bodies of water.

**Computation of Water Body Volume:** To determine volume of any given body of water, make a series of transects across the body of water taking depths at regular intervals. Add the depths and divide by the number of measurements made to determine the average depth. Multiply this average depth by total surface area in order to determine the volume to be treated. Volume is expressed as acre-feet (AF) or cubic meters (m<sup>3</sup>). Surface area can be determined by Global Positioning System (GPS) instrumentation and topographic maps. See Rotenone SOP Manual for further guidance.

**Amount of CFT Legumine Fish Toxicant Needed for Specific Uses:** To determine the approximate number of gallons (or liters) needed, find

your "Type of Use" in the first column of the tables below and then divide the corresponding numbers in the fourth column, "AF (or m<sup>3</sup>) per Gallon (or Liter) Liquid" into the number of AF or m<sup>3</sup> in your body of water. For example, a normal use of 0.05 ppm active rotenone will require 33 gallons of 5% active rotenone liquid for 100 AF.

**Table – Recommended rotenone treatment concentrations and number of acre-feet (AF) standing water covered by one gallon (5% A.I.) product.** Adjust amount of product according to the actual rotenone content on Ingredient Statement on label.

Type of Use	Parts per Million (ppm)		AF Per Gallon Liquid
	Product (5% A.I.)	Active Rotenone	
Normal	0.5 – 1.0	0.025 – 0.05	6.0 to 3.0
Tolerant Species	1.0 – 3.0	0.05 – 0.15	3.0 to 1.0
Tolerant Species in Organic Ponds	2.0 – 4.0	0.10 – 0.20	1.5 to 0.75

**Table – Recommended rotenone treatment concentrations and number of cubic meters (m<sup>3</sup>) standing water covered by one liter of (5% A.I.) product.** Adjust amount of product according to the actual rotenone content on Ingredient Statement on label.

Type of Use	Parts per Million (ppm)		m <sup>3</sup> per Liter Liquid
	Product (5% A.I.)	Active Rotenone	
Normal	0.5 – 1.0	0.025 – 0.05	2000 to 1000
Tolerant Species	1.0 – 3.0	0.05 – 0.15	1000 to 333
Tolerant Species in Organic Ponds	2.0 – 4.0	0.10 – 0.20	500 to 250

**Recommended Pre-Mixing and Method of Application:** Pre-mix with water at a rate of 10% of product to site water. Uniformly apply over water surface or through underwater lines. Divide water body into manageable sections, delineated by marker buoys or flags or GPS coordinates, and treat within 48 hours to avoid deactivation. See Rotenone SOP Manual (SOP 8) for additional guidance.

**Deactivation:** Water treated with this product will deactivate (neutralize) under natural conditions within one week to one month depending upon temperatures, alkalinity, etc. Rapid deactivation can be accomplished by adding potassium permanganate to the water at the same rate as CFT Legumine Fish Toxicant in parts per million, plus enough additional to meet the organic demand of the untreated water. See Rotenone SOP Manual (SOP 6 and 7) for guidance.

**Restocking after Treatment:** Typically, wait 2 to 4 weeks after treatment prior to restocking. Place a sample of fish to be stocked in wire cages in the coolest part of the treated waters. If the fish are not killed within 24 hours, the water may be restocked.

### USE IN STREAMS AND RIVERS

In order to treat a stream you must: (1) Select the concentration of active rotenone; (2) Compute the flow rate of the stream; (3) Select an exposure time; (4) Select dilution of product and calculation of application rate; (5) Estimate the amount of product needed; and (6) Follow the method of application. For practicality, flows > 25 ft<sup>3</sup>/s (> 0.708 m<sup>3</sup>/s) should have undiluted product applied, and flows < 25 ft<sup>3</sup>/s (< 0.708 m<sup>3</sup>/s) should have diluted product applied. For streams associated with a treatment of a standing body of water, to prevent movement of fish from the pond, lake, or reservoir, the stream treatment should begin before and continue throughout treatment of the pond, lake or reservoir until mixing has occurred.

#### Concentration of Active Rotenone

Select the concentration of active rotenone based on the type of use from those listed on the tables on the next page. Example: If you select

-continued-

"normal use", you could select a concentration of 0.025–0.05 parts per million.

**Table** – Recommended rotenone treatment concentrations and number of cubic feet per second (ft<sup>3</sup>/s) flowing water treated for 4- and 8-hour periods with one gallon of (5% A.I.) product. Adjust amount of product according to the actual rotenone content on Ingredient Statement on label.

Type of Use	Parts per Million (ppm)		ft <sup>3</sup> /s per Gallon (4-hr)	ft <sup>3</sup> /s per Gallon (8-hr)
	Product (5% A.I.)	Active Rotenone		
Normal	0.5 – 1.0	0.025 – 0.05	18.4 to 9.2	9.2 to 4.6
Tolerant Species	1.0 – 3.0	0.05 – 0.15	9.2 to 3.1	4.6 to 1.6
Tolerant Species in Organic Waters	2.0 – 4.0	0.10 – 0.20	4.6 to 2.3	2.3 to 1.2

**Table** – Recommended rotenone treatment concentrations and number of cubic meters per second (m<sup>3</sup>/s) flowing water treated for 4- and 8-hour periods with one liter of (5% A.I.) product. Adjust amount of product according to the actual rotenone content on Ingredient Statement on Label.

Type of Use	Parts per Million (ppm)		m <sup>3</sup> /s per Liter (4-hr)	m <sup>3</sup> /s per Liter (8-hr)
	Product (5% A.I.)	Active Rotenone		
Normal	0.5 – 1.0	0.025 – 0.05	0.138 to 0.069	0.069 to 0.034
Tolerant Species	1.0 – 3.0	0.05 – 0.15	0.069 to 0.024	0.034 to 0.013
Tolerant Species in Organic Waters	2.0 – 4.0	0.10 – 0.20	0.034 to 0.018	0.018 to 0.008

#### Measurement of Flow Rate for Stream

Select a cross section of the stream where the banks and bottom are relatively smooth and free of obstacles and the flow appears laminar. Best discharge measurements are achieved with an electronic flow meter and use of the United States Geological Survey *Weighted Area Method*. Alternatively, divide the stream surface width into 3 equal sections and determine the water depth and surface velocity at the center of each section. Determine the velocity by dropping a float and measure the time required to move 10 feet or more. Take at least three readings at each point. To calculate the flow rate from the information obtained above, use the following formula:

$$F = \frac{Ws \times D \times L \times C}{T}$$

Where F = flow rate (ft<sup>3</sup>/s or m<sup>3</sup>/s), Ws = surface width (ft or m), D = mean depth (ft or m), L = mean distance traveled by float (ft or m), C = Constant (0.8 for rough bottoms and 0.9 for smooth bottoms), T = mean time (s) for float to travel distance.

#### Exposure Time and Spacing

Apply rotenone as a drip for 4 to 8 hours to the flowing portion of the stream. Multiple application sites are used along the length of the treated stream, spaced approximately ½ to 2 miles apart depending on the water flow travel time between sites. Multiple sites are used because rotenone is diluted and detoxified with distance. Application sites are spaced at no more than 2 hours or at no less than 1-hour travel time intervals. This assures that the treated stream remains lethal to fish for a minimum of 2 hours. A non-toxic dye such as Rhodamine-WT or fluorescein can be used to determine travel times. Cages containing

live fish placed immediately upstream of the downstream application sites can be used as sentinels to assure that lethal conditions exist between sites.

#### Amount of Product and Calculation of Application Rate of Undiluted Product:

$$X = F1 (1.699 B) \text{ or } X = F2 (59.99 B)$$

X = ml per minute of undiluted CFT Legumine Fish Toxicant applied to the stream, F1 = the flow rate (ft<sup>3</sup>/s) and F2 the flow rate (m<sup>3</sup>/s) (see Measurement of Flow Rate for Stream on this labeling), B = parts per million desired concentration of CFT Legumine Fish Toxicant. Total amount of product needed:

$$Y = X(60)H$$

Y = total ml of undiluted CFT Legumine Fish Toxicant required for treatment, X = ml per minute of undiluted product, and H = duration (hours) of treatment.

#### Amount of Product in Drip Can and Flow Rate of Diluted Product:

$$Y = B(102 F1)H \text{ or } Y = B(3,602 F2)H$$

Y = ml of undiluted product in the reservoir, B = parts per million desired concentration of CFT Legumine Fish Toxicant, F1 = the flow rate (ft<sup>3</sup>/s) and F2 = flow rate (m<sup>3</sup>/s) (see Measurement of Flow Rate for Stream in this labeling), and H = duration (hours) of treatment.

Discharge of the diluted product:

$$X = Z/60/H$$

X = ml per minute of diluted CFT Legumine Fish Toxicant applied to the stream from drip can, Z = volume (ml) of drip can, and H = duration (hours) of treatment.

#### Method of Application

The unique nature of every application site could require minor adjustments to the method and rate of application. Should these unique conditions require major deviation from the use directions, a Special Local Need 24(c) registration should be obtained from the state. Before application, authorization must be obtained from state or federal Fish and Wildlife and/or Natural Resource agencies. Since local environmental conditions will vary, consult with the state Fish and Wildlife and/or Natural Resource agency to ensure the method and rate of application are appropriate for that site.

Contact the local water department to determine if any water intakes are within one mile downstream of the section of stream, river, or canal to be treated. If so, coordinate the application with the water department to make sure the intakes are closed during treatment and detoxification.

CFT Legumine Fish Toxicant can drain directly into the center of the stream. Flow should be checked at least hourly. Backwater, stagnant, and spring areas of streams should be sprayed by hand with a 1 to 2 % v/v solution of 5% rotenone product to assure complete coverage. Streams should be treated for 4 to 8 hours in order to clear the treated section of stream of fish. See Rotenone SOP Manual for detailed guidance on application equipment, methods, and strategies.

#### DEACTIVATION

Flow in a stream and outflow from a treated lake beyond the treatment area must be deactivated with potassium permanganate to minimize exposure beyond the treatment area unless unnecessary. (See Rotenone SOP Manual [SOP 6] for the definition of treatment area, examples when deactivation with potassium permanganate is unnecessary and detailed guidance for deactivating with potassium permanganate [SOP 7].)

Within 1 to 2 hours travel time from the furthest downstream rotenone application site, the rotenone can be deactivated with a potassium

-continued-



permanganate solution or granules at a resultant stream concentration of 2 to 4 parts per million, depending on rotenone concentration and organic demand of the water. A 2.5% (10 pounds potassium permanganate to 50 gallons of water) permanganate solution is dripped in at a continuous rate using the equation:

$$X = Y(70 F1) \text{ or } X = Y(2,472 F2)$$

X = ml of 2.5% permanganate solution per minute, Y = ppm of desired permanganate concentration, F1 = stream flow (ft<sup>3</sup>/s) or F2 = stream flow (m<sup>3</sup>/s) or, granular potassium permanganate is applied at a continuous rate using the equations:

$$Z = Y(1.7 F1) \text{ or } Z = Y(60.02 F2)$$

Z = grams of granular potassium permanganate per minute, Y = ppm of desired permanganate concentration, F1 = stream flow (ft<sup>3</sup>/s) or F2 = stream flow (m<sup>3</sup>/s).

Flow of permanganate should be checked at least hourly. Live fish in cages placed immediately above the permanganate application site will show signs of stress signaling the need for beginning deactivation. Deactivation can be terminated when replenished fish survive and show no signs of stress for at least four hours.

Deactivation of rotenone by permanganate requires between 15 to 30 minutes contact time (travel time). Cages containing live fish can be placed at these downstream intervals to judge the effectiveness of deactivation. At water temperatures less than 50°F, deactivation may be retarded, requiring a longer contact time.

#### STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

**PESTICIDE STORAGE:** Store only in original containers, in a dry place inaccessible to children and pets. This product will not solidify nor show any separation at temperatures down to 40°F and is stable for a minimum of one year when stored in sealed drums at 70°F.

**PESTICIDE DISPOSAL:** Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal law. If these wastes cannot be disposed of by use according to label instructions, contact your state pesticide or Environmental Control Agency, or the Hazardous Waste representative at the nearest EPA Regional office for guidance.

**CONTAINER HANDLING:** Nonrefillable container. Do not reuse or refill this container. Clean container promptly after emptying.

*(For Containers equal to or less than 5 Gallons:)* Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Offer for recycling, if available or puncture and dispose of in a sanitary landfill, or by incineration, or if allowed by state and local authorities, by burning. If burned, stay out of smoke.

*(For Containers greater than 5 Gallons:)* Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water. Replace and tighten closures. Tip container on its side and roll it back and forth, ensuring at least one complete revolution, for 30 seconds. Stand the container on its end and tip it back and forth several times. Turn the container over onto its other end and tip it back and forth several times. Empty the rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Repeat this procedure two more times. Offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

#### WARRANTY STATEMENT

Our recommendations for the use of this product are based upon tests believed to be reliable. The use of this product being beyond the control of the manufacturer, no guarantee, expressed or implied, is made as to the effects of such or the results to be obtained if not used in accordance with directions or established safe practice. To the extent consistent with applicable law, the buyer must assume all responsibility, including injury or damage, resulting from its misuse as such, or in combination with other materials.

Circled letter in front of the EPA Est No. corresponds to the first letter in lot number on bottom of container.

**Central Garden & Pet Company**, 1501 East Woodfield Road, 200W, Schaumburg, Illinois 60173

**NOTE:** This specimen label is for informational purposes only. All uses may not be approved in all states. See product labeling for use directions.

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Schaumburg, IL





## **APPENDIX C: TARGET BOAT SPEEDS FOR ROTENONE APPLICATION**

Appendix C1.—Target boat speeds for applying CFT Legumine at select lake depths to attain about 40 ppb rotenone concentration.

Water depth (ft.)	Water volume <sup>a</sup>	Gallons of product <sup>b</sup>	Boat speed (mph) <sup>c</sup>
1	0.07	0.0	46.4
2	0.14	0.0	23.2
3	0.21	0.1	15.5
4	0.28	0.1	11.6
5	0.34	0.1	9.3
6	0.41	0.1	7.7
7	0.48	0.1	6.6
8	0.55	0.1	5.8
9	0.62	0.2	5.2
10	0.69	0.2	4.6
11	0.76	0.2	4.2
12	0.83	0.2	3.9
13	0.90	0.2	3.6
14	0.96	0.3	3.3
15	1.03	0.3	3.1
16	1.10	0.3	2.9
17	1.17	0.3	2.7
18	1.24	0.3	2.6
19	1.31	0.3	2.4
20	1.38	0.4	2.3
21	1.45	0.4	2.2
22	1.52	0.4	2.1
23	1.58	0.4	2.0
24	1.65	0.4	1.9
25	1.72	0.5	1.9
26	1.79	0.5	1.8
27	1.86	0.5	1.7
28	1.93	0.5	1.7
29	2.00	0.5	1.6
30	2.07	0.6	1.5

*Note:* Target treatment concentration was 0.80 ppm of rotenone product (40 ppb rotenone). It was assumed that the boat could apply 0.75 gallons of liquid formulation per minute.

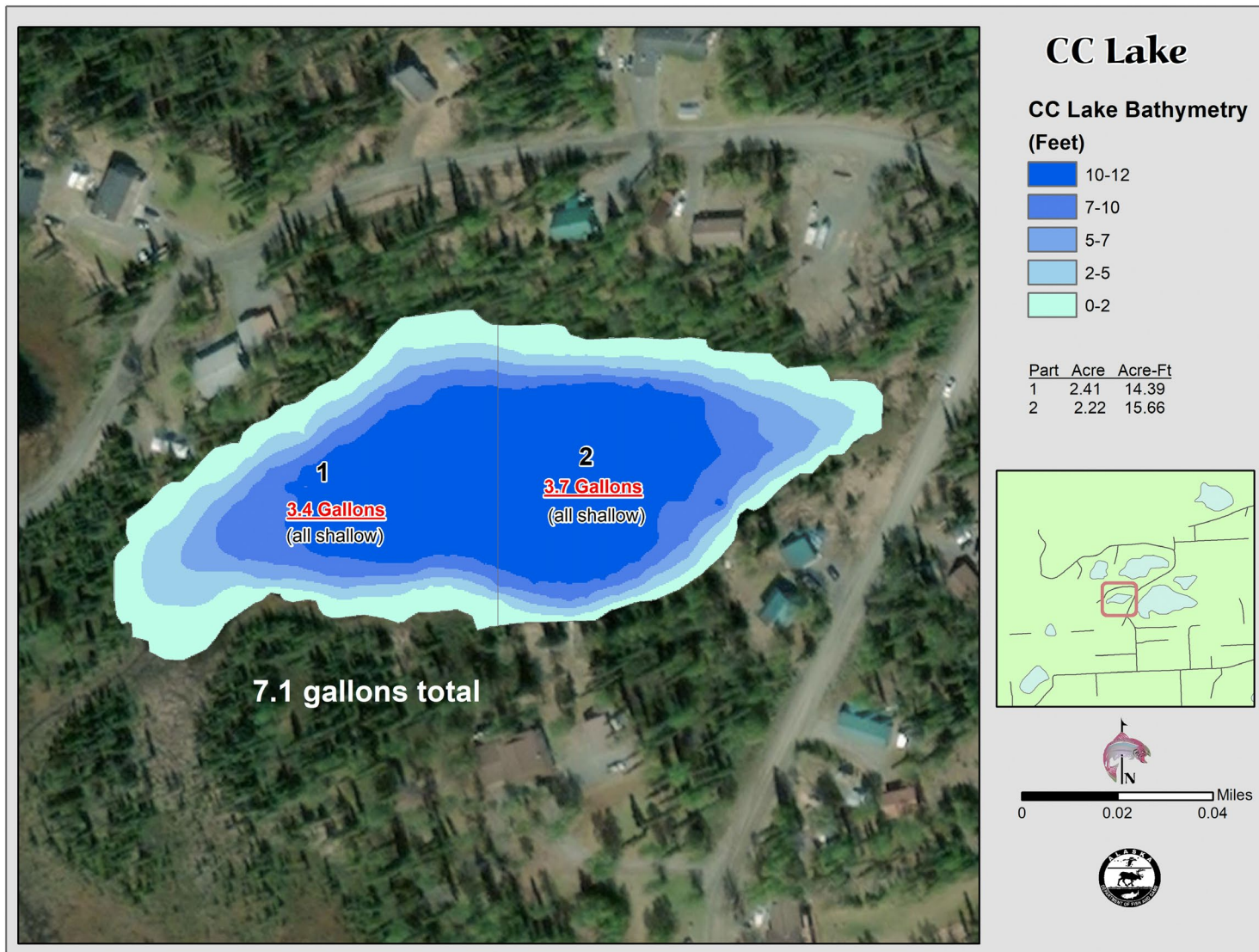
<sup>a</sup> Water volume (acre-feet) in every 100 linear foot stretch of a 30 ft wide application swath.

<sup>b</sup> Gallons of product needed per 100 linear feet of boat travel to apply product at a rotenone concentration of 40 ppb.

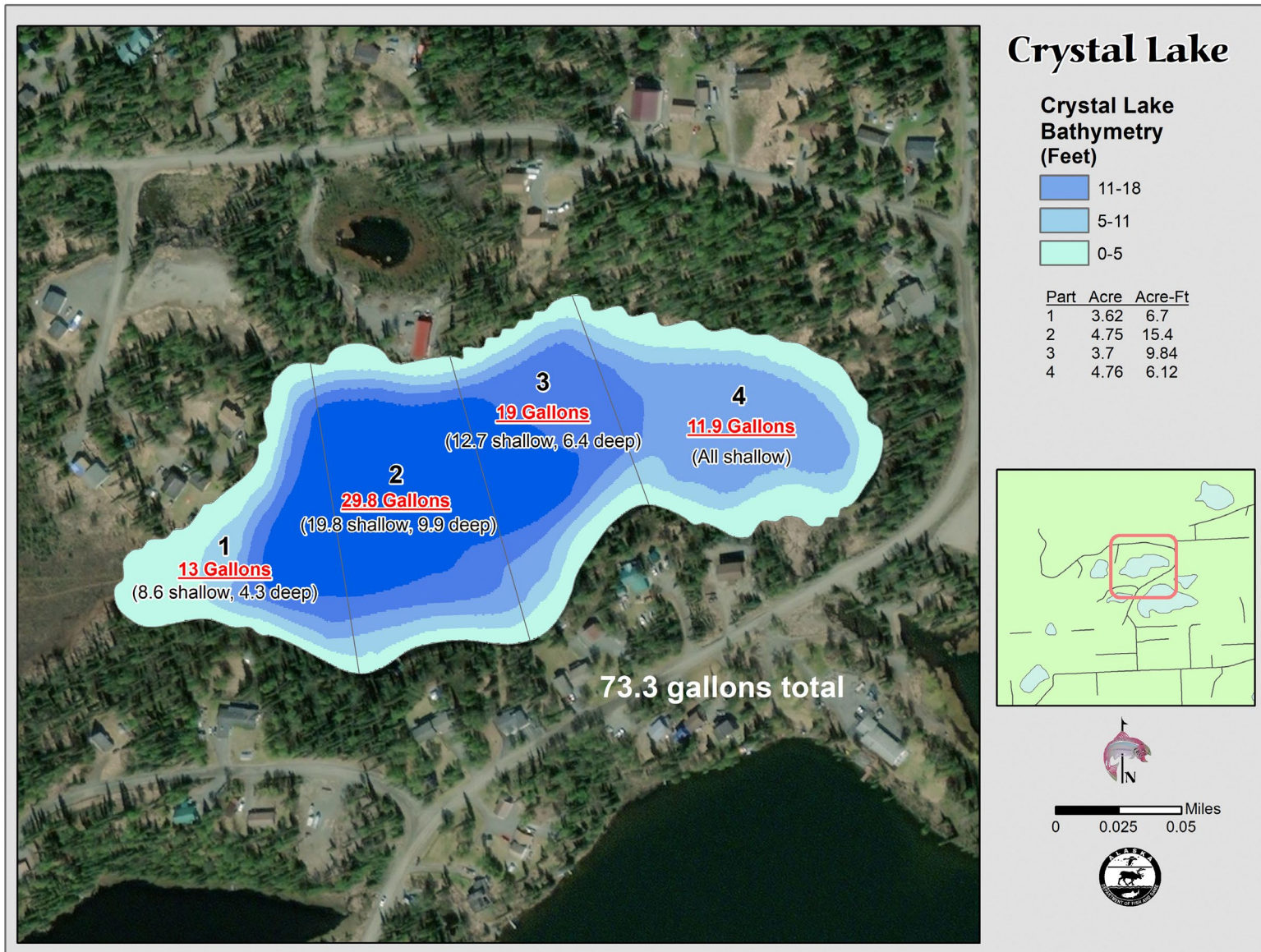
<sup>c</sup> Boat speed is in miles per hour. At water depths <5 ft, target boat speed is impractically fast, applicators would reduce product pumping rate so boat speed could be reduced accordingly to a feasible speed.

## **APPENDIX D: BATHYMETRY MAPS**

Appendix D1.—Bathymetric map of CC Lake showing amount and depth of CFT Legumine application to each section.

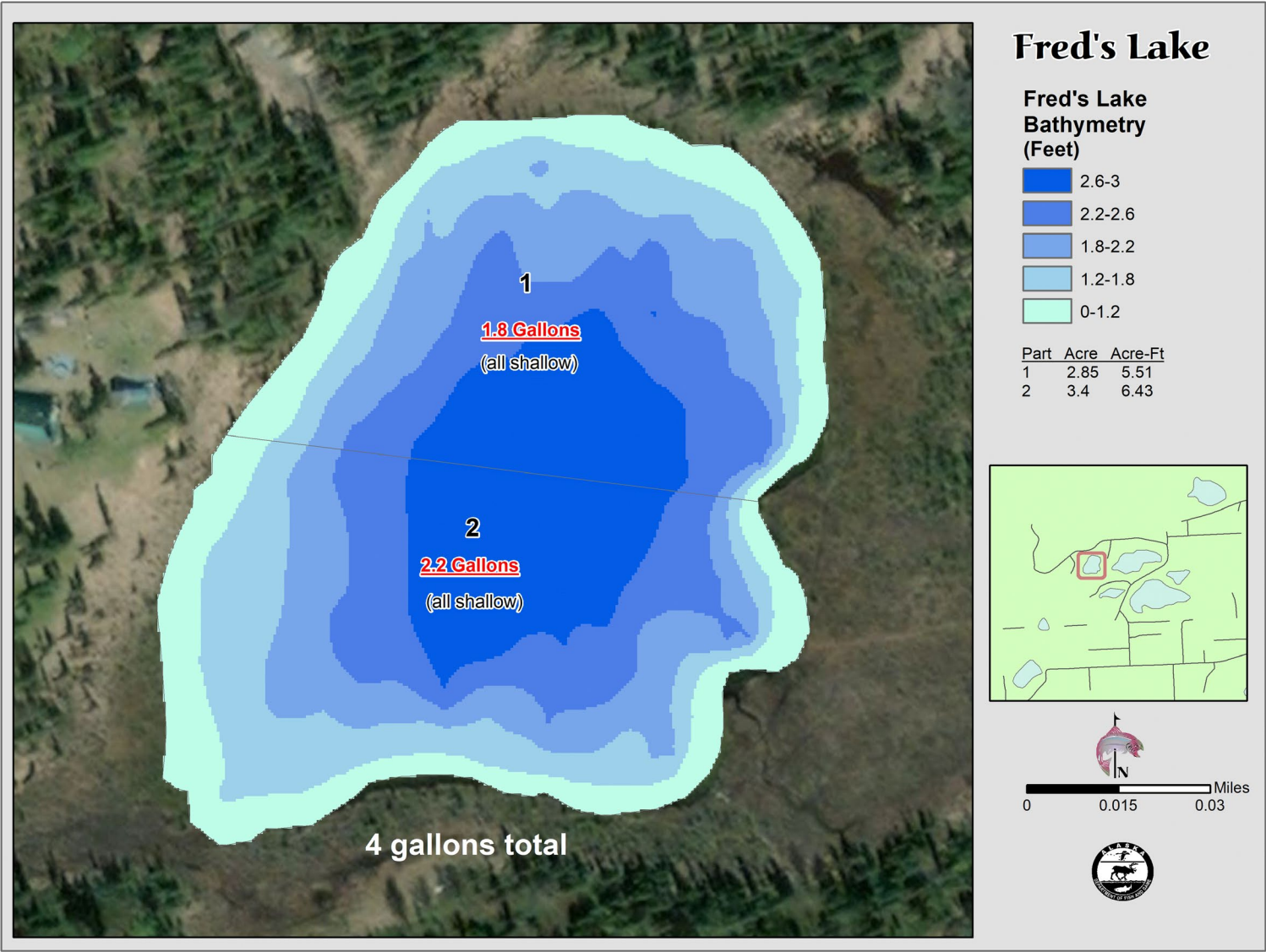


Appendix D2.—Bathymetric map of Crystal Lake showing amount and depth of CFT Legumine application to each section.



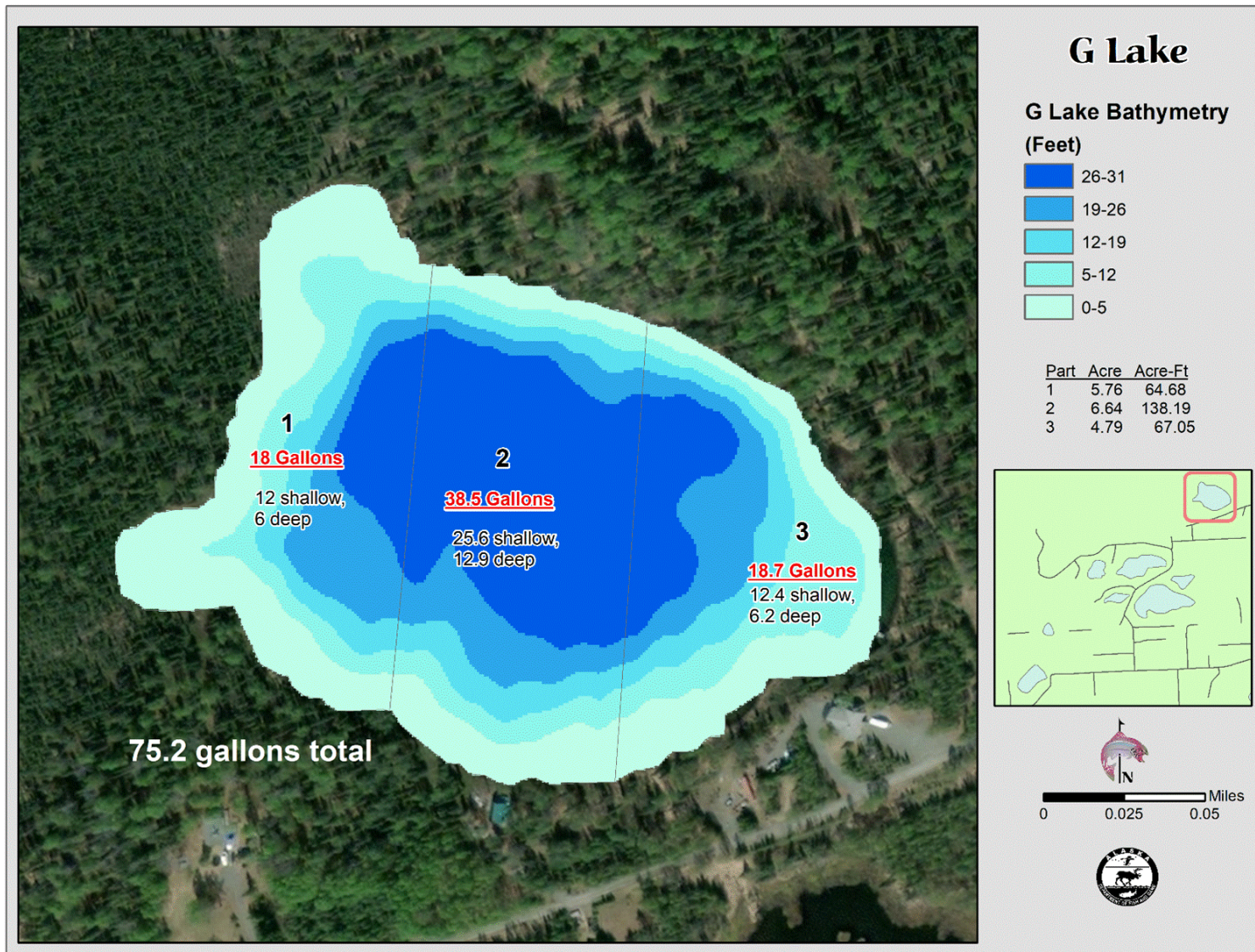


Appendix D3.—Bathymetric map of Freds Lake showing amount and depth of CFT Legumine application to each section.



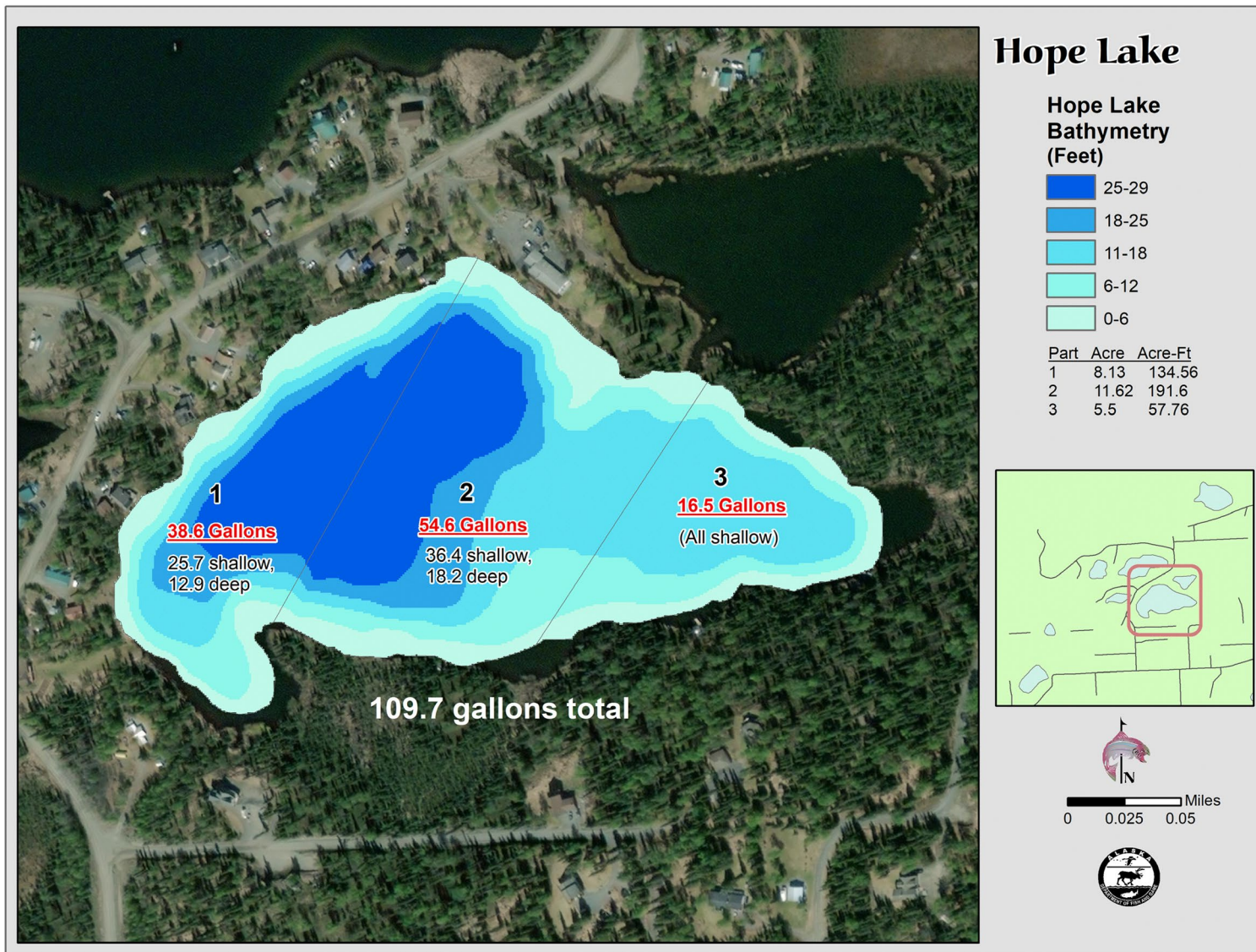


Appendix D4.—Bathymetric map of G Lake showing amount and depth of CFT Legumine application to each section.



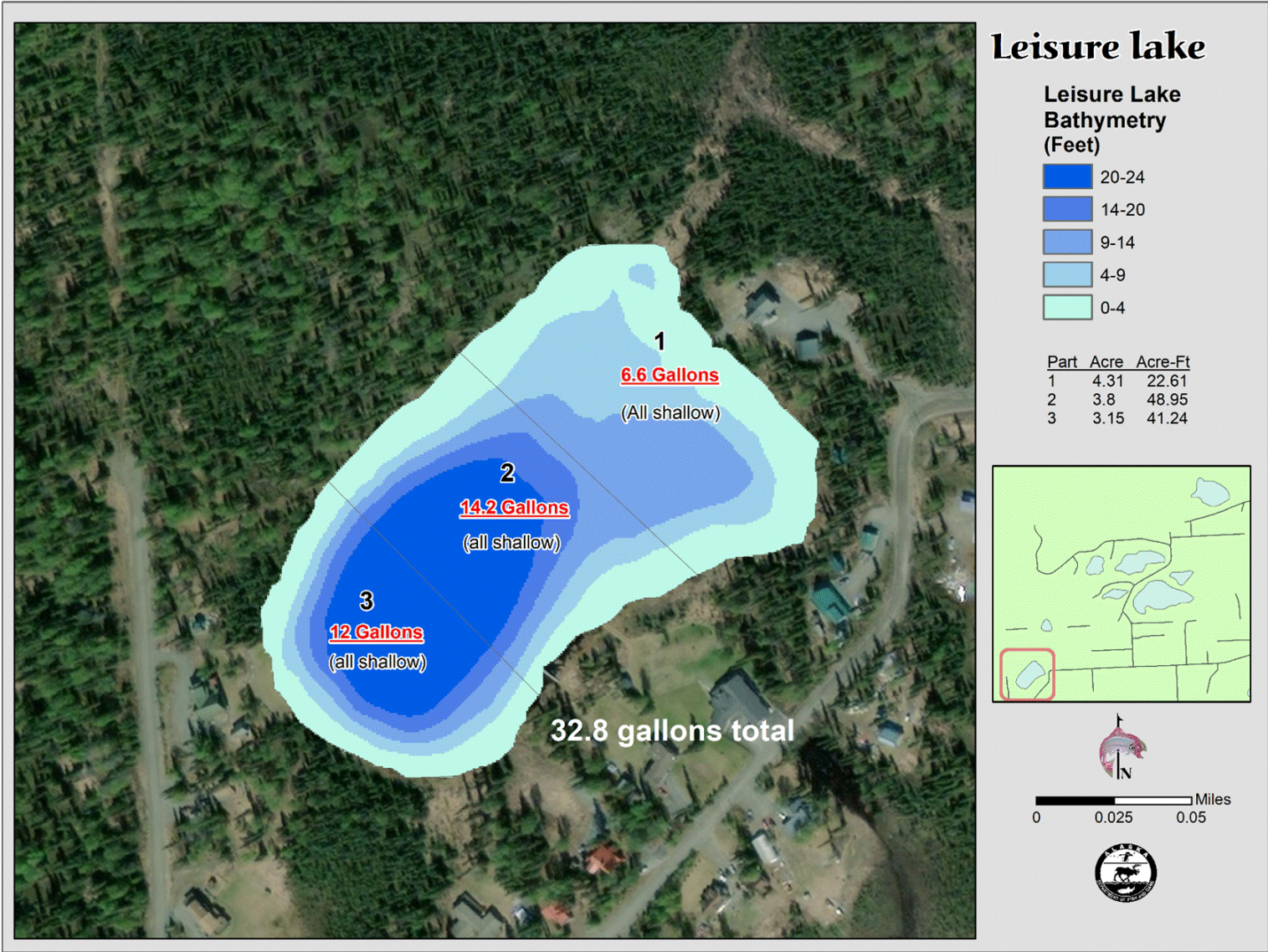


Appendix D5.—Bathymetric map of Hope Lake showing amount and depth of CFT Legumine application to each section.



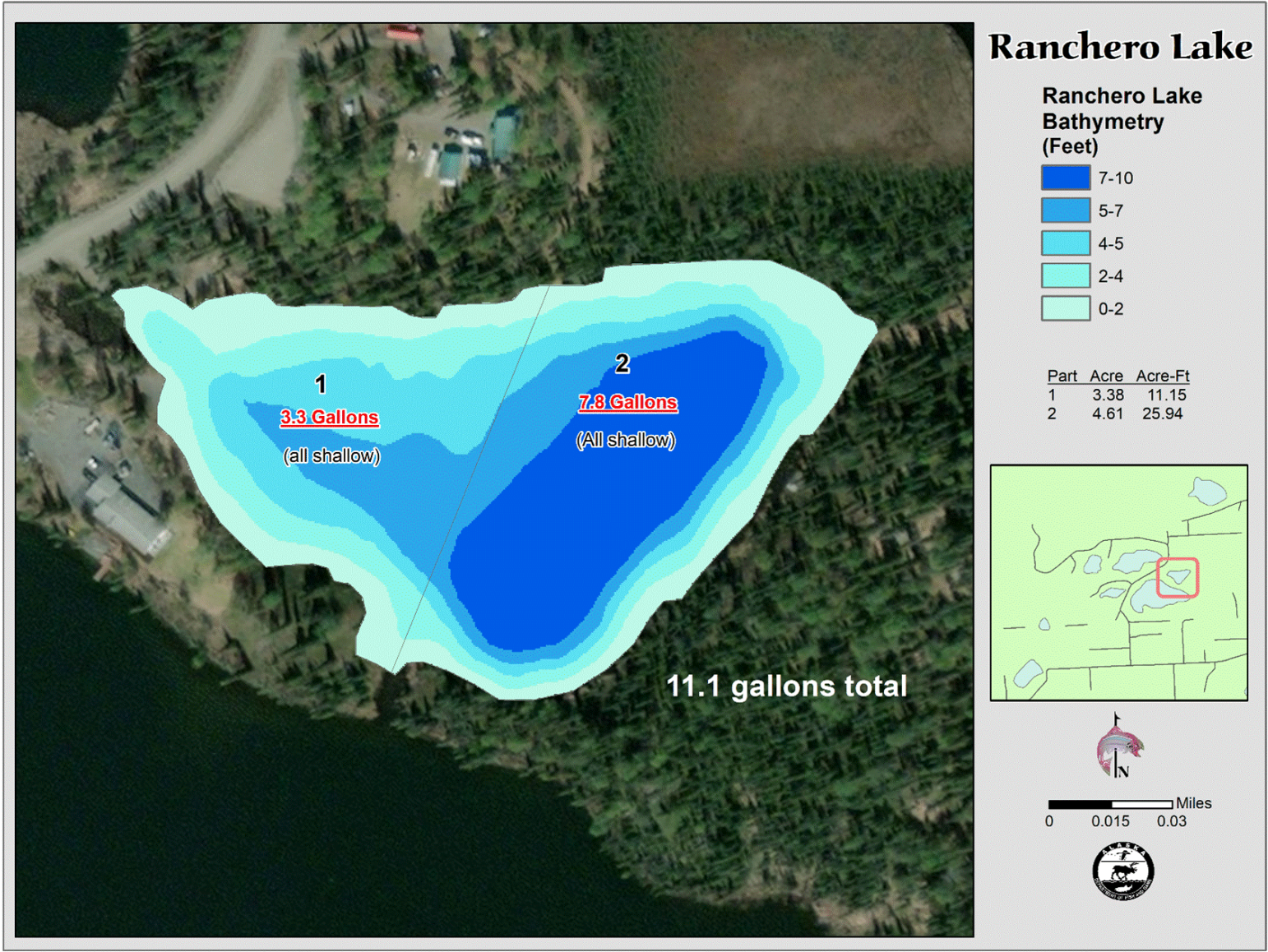


Appendix D6.—Bathymetric map of Leisure Lake showing amount and depth of CFT Legumine application to each section.





Appendix D7.—Bathymetric map of Ranchero Lake showing amount and depth of CFT Legumine application to each section.



**APPENDIX E: FORK LENGTHS AND SEX OF CAPTURED  
NORTHERN PIKE AND MUSKELLUNGE**

Appendix E1.–Fork lengths (FL) and sex identification of Tote Road Lakes area northern pike captured in gillnets, 8 May 2018–12 October 2018.

Lake	Date	Fork length (mm)	Sex
CC Lake	15 Aug	219	U
	15 Aug	228	U
	15 Aug	240	F
	15 Aug	287	F
	15 Aug	294	M
	15 Aug	298	F
	15 Aug	302	U
	15 Aug	303	M
	15 Aug	317	F
	15 Aug	329	M
	15 Aug	331	M
	15 Aug	335	M
	15 Aug	353	U
	15 Aug	360	M
	15 Aug	363	F
	15 Aug	365	F
	15 Aug	370	M
	15 Aug	383	M
	15 Aug	825	F
	16 Aug	217	U
	16 Aug	218	U
	16 Aug	226	U
	16 Aug	285	M
	16 Aug	302	M
	16 Aug	307	F
	16 Aug	312	M
	16 Aug	318	M
	16 Aug	337	F
	16 Aug	345	F
	16 Aug	373	M
	16 Aug	386	F
	16 Aug	387	M
	16 Aug	404	F
	16 Aug	405	M
	16 Aug	458	M
	20 Aug	193	U
	20 Aug	256	U
	20 Aug	303	M
	20 Aug	317	U
	20 Aug	362	F
	20 Aug	362	M
	20 Aug	372	F
	20 Aug	406	F
	20 Aug	451	M

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Lake	Date	Fork length (mm)	Sex
Crystal Lake	21 Aug	287	F
	21 Aug	327	M
	21 Aug	357	M
	21 Aug	402	M
	21 Aug	422	M
	21 Aug	437	M
	21 Aug	438	M
	21 Aug	447	M
	21 Aug	454	M
	21 Aug	460	F
	21 Aug	469	M
	21 Aug	475	F
	21 Aug	528	F
	21 Aug	548	F
	22 Aug	205	U
	22 Aug	209	M
	22 Aug	221	U
	22 Aug	226	U
	22 Aug	246	M
	22 Aug	276	F
	22 Aug	281	M
	22 Aug	284	M
	22 Aug	302	F
	22 Aug	312	M
	22 Aug	314	F
	22 Aug	321	M
	22 Aug	323	M
	22 Aug	329	F
	22 Aug	352	M
	22 Aug	371	F
	22 Aug	423	M
	22 Aug	425	M
	22 Aug	458	M
	22 Aug	461	M
	22 Aug	570	M
	23 Aug	183	U
	23 Aug	278	U
	23 Aug	279	M
	23 Aug	280	F
	23 Aug	280	M
	23 Aug	280	U
	23 Aug	300	M
	23 Aug	306	F
	23 Aug	322	F
	23 Aug	323	M
	23 Aug	333	F

-continued-

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Lake	Date	Fork length (mm)	Sex
Crystal Lake (continued)	23 Aug	333	M
	23 Aug	340	M
	23 Aug	402	M
	23 Aug	436	M
	23 Aug	469	M
	23 Aug	490	U
	23 Aug	501	M
	23 Aug	503	F
	27 Aug	510	F
	27 Aug	216	U
	27 Aug	233	M
	27 Aug	241	M
	27 Aug	255	F
	27 Aug	267	M
	27 Aug	282	M
	27 Aug	285	M
	27 Aug	287	F
	27 Aug	289	M
	27 Aug	291	F
	27 Aug	291	M
	27 Aug	292	U
	27 Aug	294	M
	27 Aug	301	U
	27 Aug	302	M
	27 Aug	309	M
	27 Aug	312	F
	27 Aug	326	M
	27 Aug	338	M
	27 Aug	372	F
	27 Aug	374	F
	27 Aug	388	M
	27 Aug	412	M
	27 Aug	415	M
	27 Aug	426	M
	27 Aug	436	M
	27 Aug	451	M
	27 Aug	460	M
	27 Aug	475	M
	27 Aug	521	M
	27 Aug	222	M
	27 Aug	225	M
	27 Aug	293	F
	27 Aug	295	M
	27 Aug	297	M
	27 Aug	304	M
	27 Aug	321	M

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Lake	Date	Fork length (mm)	Sex
Crystal Lake (continued)	27 Aug	328	M
	27 Aug	329	F
	27 Aug	355	M
	27 Aug	433	F
	27 Aug	462	M
	27 Aug	467	M
	27 Aug	481	M
	27 Aug	490	M
	29 Sep	314	M
Hope Lake	2 Oct	250	U
	2 Oct	252	U
	2 Oct	270	U
	2 Oct	273	U
	2 Oct	278	U
	2 Oct	280	U
	2 Oct	302	U
	2 Oct	303	U
	2 Oct	308	U
	2 Oct	312	U
	2 Oct	345	U
	2 Oct	365	U
	2 Oct	377	U
	2 Oct	404	U
	2 Oct	406	U
	2 Oct	421	U
	2 Oct	421	U
	2 Oct	422	U
	2 Oct	431	U
	2 Oct	440	U
	2 Oct	460	U
	2 Oct	479	U
	2 Oct	523	U
	2 Oct	577	U
	3 Oct	220	U
	3 Oct	228	U
	3 Oct	232	U
	3 Oct	235	U
	3 Oct	248	U
	3 Oct	355	U
	3 Oct	364	U
	3 Oct	380	U
	3 Oct	404	U
	3 Oct	430	U
	3 Oct	440	U
	3 Oct	471	U

-continued-

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Lake	Date	Fork length (mm)	Sex
Leisure Lake	13 Aug	237	F
	13 Aug	248	M
	13 Aug	261	M
	13 Aug	268	M
	13 Aug	280	F
	13 Aug	280	
	13 Aug	282	F
	13 Aug	286	M
	13 Aug	288	M
	13 Aug	297	F
	13 Aug	300	F
	13 Aug	301	M
	13 Aug	310	M
	13 Aug	318	F
	13 Aug	320	F
	13 Aug	320	M
	13 Aug	322	F
	13 Aug	327	M
	13 Aug	330	F
	13 Aug	331	M
	13 Aug	332	U
	13 Aug	340	M
	13 Aug	350	F
	13 Aug	361	F
	13 Aug	362	F
	13 Aug	741	F
	14 Aug	252	M
	14 Aug	264	M
	14 Aug	277	M
	14 Aug	297	M
	14 Aug	305	M
	14 Aug	315	F
	14 Aug	317	F
	14 Aug	320	F
	14 Aug	323	M
	14 Aug	338	F
	14 Aug	340	F
	14 Aug	349	F
	14 Aug	355	F
	14 Aug	359	F
Ranchero Lake	8 May	441	M
	8 May	471	M
	8 May	502	M
	8 May	476	M
	8 May	475	M
	8 May	424	M

-continued-



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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	8 May	331	M
	8 May	340	M
	8 May	477	M
	8 May	322	M
	8 May	320	M
	8 May	505	M
	8 May	489	M
	8 May	520	M
	8 May	308	M
	8 May	390	M
	8 May	560	M
	8 May	490	M
	8 May	321	M
	8 May	357	M
	8 May	430	M
	8 May	430	M
	8 May	345	M
	8 May	456	M
	8 May	410	M
	8 May	410	M
	8 May	455	M
	8 May	411	M
	8 May	512	M
	8 May	461	M
	8 May	343	M
	8 May	404	M
	8 May	377	M
	8 May	421	M
	8 May	454	M
	8 May	528	F
	8 May	532	F
	8 May	460	F
	8 May	421	F
	8 May	397	F
	8 May	380	F
	8 May	381	M
	8 May	383	M
	8 May	275	U
	8 May	396	M
	8 May	383	M
	8 May	331	M
	8 May	320	M
	8 May	335	M
	8 May	471	M
	8 May	326	M
	8 May	444	M

-continued-

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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	8 May	458	M
	8 May	323	M
	8 May	464	M
	8 May	296	M
	8 May	389	M
	8 May	531	F
	8 May	494	F
	8 May	440	F
	8 May	493	F
	8 May	375	F
	8 May	368	F
	8 May	366	F
	8 May	343	M
	8 May	345	M
	8 May	357	M
	8 May	361	M
	8 May	346	M
	8 May	295	M
	8 May	288	M
	8 May	511	M
	8 May	500	M
	8 May	616	M
	8 May	472	F
	8 May	533	M
	8 May	445	M
	8 May	541	M
	8 May	421	M
	8 May	361	M
	8 May	469	M
	8 May	343	M
	8 May	412	M
	8 May	313	M
	8 May	355	M
	8 May	384	M
	8 May	485	M
	8 May	441	M
	8 May	450	M
	8 May	350	M
	8 May	373	M
	8 May	474	M
	8 May	335	M
	8 May	416	M
	8 May	532	M
	8 May	535	M
	8 May	510	M
	8 May	487	M

-continued-

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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	8 May	440	M
	8 May	470	M
	8 May	545	F
	8 May	495	U
	8 May	463	U
	8 May	380	U
	8 May	285	U
	8 May	375	U
	8 May	311	U
	8 May	321	U
	8 May	360	U
	8 May	268	U
	8 May	325	U
	8 May	291	U
	8 May	304	U
	8 May	376	U
	8 May	320	U
	8 May	252	U
	8 May	478	U
	8 May	419	U
	8 May	416	U
	8 May	389	U
	8 May	419	U
	8 May	420	U
	8 May	353	U
	8 May	363	U
	8 May	357	U
	8 May	370	U
	8 May	340	U
	8 May	445	U
	8 May	348	U
	8 May	367	U
	8 May	401	U
	8 May	306	U
	8 May	420	U
	8 May	294	U
	8 May	332	U
	8 May	305	U
	8 May	419	U
	8 May	322	U
	8 May	320	U
	8 May	457	U
	8 May	304	U
	8 May	292	U
	8 May	357	U
	8 May	316	U

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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	8 May	338	U
	8 May	286	U
	8 May	308	U
	8 May	354	U
	8 May	305	U
	8 May	322	U
	8 May	320	U
	8 May	312	U
	8 May	363	U
	8 May	246	U
	8 May	272	U
	8 May	325	U
	8 May	326	U
	8 May	293	U
	8 May	347	U
	8 May	253	U
	8 May	374	U
	8 May	349	U
	8 May	252	U
	8 May	241	U
	8 May	317	U
	8 May	355	U
	8 May	286	U
	8 May	315	U
	8 May	324	U
	8 May	255	U
	8 May	244	U
	8 May	291	U
	8 May	256	U
	8 May	268	U
	8 May	342	U
	8 May	280	U
	8 May	224	U
	8 May	241	U
	8 May	465	U
	8 May	385	U
	8 May	417	U
	8 May	420	U
	8 May	310	U
	8 May	375	U
	8 May	484	U
	8 May	333	U
	8 May	280	U
	8 May	318	U
	8 May	291	U
	8 May	285	U

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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	8 May	311	U
	8 May	306	U
	18 Sep	338	M
	18 Sep	261	M
	18 Sep	258	U
	18 Sep	251	F
	18 Sep	400	M
	18 Sep	249	U
	18 Sep	346	F
	18 Sep	314	F
	18 Sep	240	F
	18 Sep	332	F
	18 Sep	371	M
	18 Sep	440	F
	18 Sep	456	F
	18 Sep	494	F
	18 Sep	410	F
	18 Sep	536	F
	18 Sep	454	F
	18 Sep	411	U
	18 Sep	336	M
	18 Sep	338	M
	18 Sep	261	U
	18 Sep	326	M
	18 Sep	320	F
	18 Sep	235	M
	18 Sep	345	M
	19 Sep	605	F
	19 Sep	402	M
	19 Sep	424	M
	19 Sep	456	F
	19 Sep	357	M
	19 Sep	389	M
	19 Sep	388	M
	19 Sep	340	M
	19 Sep	314	M
	19 Sep	331	M
	19 Sep	299	M
	19 Sep	325	U
	19 Sep	268	U
	19 Sep	275	M
	19 Sep	228	U
	19 Sep	211	F
	19 Sep	297	M
	19 Sep	245	U
	20 Sep	535	F

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Lake	Date	Fork length (mm)	Sex
Ranchero Lake (continued)	20 Sep	337	F
	20 Sep	237	F
	20 Sep	554	M
	20 Sep	396	M
	20 Sep	582	M
	20 Sep	342	F
	20 Sep	400	U
	20 Sep	381	U
	20 Sep	366	U
	20 Sep	324	U
	20 Sep	309	U
	20 Sep	231	U
	20 Sep	265	U
	20 Sep	320	U
	20 Sep	331	U
	20 Sep	271	U
	20 Sep	266	U
	20 Sep	241	U
	20 Sep	296	U
	20 Sep	252	U
	20 Sep	289	U
	20 Sep	219	U

*Note:* M = male; F = female; U = unknown.

Appendix E2.–Fork lengths (FL) of G Lake muskellunge captured in gillnets, 2017–2018.

Lake	Date (M/D/Y)	Fork length (mm)	Sex
G Lake	09/01/17	587	U
G Lake	09/27/17	795	U
G Lake	09/27/17	775	U
G Lake	09/27/17	405	U
G Lake	06/18/18	745	U
G Lake	06/18/18	780	U
G Lake	06/18/18	598	U
G Lake	10/12/18	U	U

*Note:* “U” means unknown.





## **APPENDIX F: UNIVERSITY OF QUEBEC INVERTEBRATE SAMPLING RESULTS**

Appendix F1.–Pretreatment invertebrate surveys of Tote Road Lakes (TRL) lakes conducted by researchers from University of Quebec, June 2018.

Order/Family	Pretreatment invertebrate taxa counts by lake								Grand total	Taxa diversity <sup>a</sup>
	CC	Crystal	Freds	G	Hope	Leisure Lake	Leisure Pond	Ranchero		
Annelida/Hirudinea	0	0	0	0	0	0	0	0	0	0
Amphipoda/Gammaridae	45	51	1	0	44	85	28	136	390	1
Annelida/Oligochaeta	0	0	0	3	0	1	1	2	7	1
Arachnida/Araneae	0	0	0	0	0	0	0	0	0	0
Annelida/Sangsue	0	0	0	0	4	0	0	0	6	1
Cladocera	1	0	21	6	0	4	3	0	35	1
Coleoptera/Chrysomelidae	0	0	0	0	0	0	0	0	0	0
Coleoptera/Dytiscidae	2	0	1	0	0	0	0	2	5	1
Coleoptera/Elmidae	0	0	0	1	0	0	0	0	2	1
Coleoptera/Gyrinidae	0	0	0	0	0	0	0	0	2	1
Coleoptera/Haliplidae	0	1	0	0	0	0	0	0	1	1
Coleoptera/Hydraenidae	0	0	0	0	0	0	0	0	0	0
Coleoptera/Hydrophilidae	0	0	0	0	0	0	0	0	0	0
Collembola/Isotomidae	0	0	0	0	0	0	0	0	0	0
Collembola/Sminthuridae	0	0	0	0	0	0	0	0	0	0
Copepoda	5	15	76		2	8	22	7	135	1
Crustacea/Ostracoda	0	0	0	0	0	0	0	0	0	0
Diptera/Ceratopogonidae	8	6	3	9	3	4	6	5	44	1
Diptera/Chironomidae	61	98	104	91	73	202	134	228	991	1
Diptera/Dixidae	0	0	0	0	0	0	0	0	0	0
Diptera/Empididae	0	0	0	0	0	2	0	0	2	1
Diptera/Nymphae	0	4	8	1	0	6	12	3	34	1
Diptera/Psychodidae	0	0	0	0	0	0	0	0	0	0
Diptera/Tabanidae	0	0	0	0	0	0	0	0	0	0
Diptera/Tipulidae	0	0	0	0	0	0	0	0	0	0
Ephemeroptera/Baetidae	0	0	0	0	0	0	0	0	0	0
Ephemeroptera/Caenidae	0	0	23	1	0	0	0	0	24	1
Ephemeroptera/Siphonuridae	0	0	1	0	0	0	0	0	1	1
Hemiptera/Aphidae	0	0	0	0	0	0	0	0	0	0
Hemiptera/Corixidae	3	1	2	0	0	0	0	0	7	1
Hydrachnidia/Hydracariens	0	0	0	0	0	0	1	1	2	1

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Order/Family	Pretreatment invertebrate taxa counts by lake								Grand total	Taxa diversity <sup>a</sup>
	CC	Crystal	Freds	G	Hope	Leisure Lake	Leisure Pond	Ranchero		
Hemiptera/Gerridae	0	0	0	0	0	0	0	0	0	0
Lepidoptera/Pyralidae	0	0	0	0	0	0	0	0	0	0
Megaloptera/Sialidae	0	0	0	0	0	0	0	0	0	0
Mollusca/Lymnaeidae	0	0	0	0	0	0	0	0	0	0
Mollusca/Planorbidae	2	8	4	1	1	12	17	7	52	1
Mollusca/Sphaeriidae	22	27		2	86	54	85	20	296	1
Mollusca/Unionides	0	0	0	0	0	0	0	0	6	1
Nematoda/Nemathelminthes	0	1	0	0	0	0	0	0	3	1
Odonata/Aeshnidae	0	2	2	1	1	0	0	6	12	1
Odonata/Anisoptera	0	0	0	0	0	1	0	0	1	1
Odonata/Calopterygidae	0	0	0	0	0	0	0	0	0	0
Odonata/Coenagrionidae	2	1	0	0	2	0	0	1	6	1
Odonata/Cordulegastridae	0	0	1	0	0	0	0	0	1	1
Odonata/Corduliidae	2	1	3	6	1	3	2	1	19	1
Odonata/Lestidae	0	0	48	0	0	3	0	7	58	1
Odonata/Libellulidae	0	0	4	0	1	0	0	2	7	1
Plecoptera/Leuctridae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Hydroptilidae	0	0	1	6	0	0	0	0	7	1
Trichoptera/Lepidostomatidae	0	1	0	0	0	0	0	0	1	1
Trichoptera/Leptoceridae	0	0	0	0	0	0	0	0	0	0
Trichoptera/limnephilidae	1	1	1	3	1	1	0	3	11	1
Trichoptera/Phylopotamidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Phryganeidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Polycentropodidae	0	0	0	0	1	1	1	0	3	1
Trichoptera/Psychomyiidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Rhyacophilidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Uenoidae	0	0	0	0	0	0	0	0	0	0
Diversity count (all lakes)										32

*Note:* All data was provided courtesy by Dr. Alison Derry and undergraduates Nathan Juilliart and Anastasiya Zuko of the University of Quebec in Montreal, Department of Biological Sciences, CP 8888, 9 Succursale Centre-Ville, Montreal QC H3C 3P8 Canada. Each lake was surveyed at 4 sites.

<sup>a</sup> Taxa diversity definition: 1 = taxon is present; 0 = taxon is not present.

Appendix F2.–Posttreatment invertebrate surveys of Tote Road Lakes (TRL) lakes conducted by researchers from University of Quebec, June 2019.

Order/Family	Posttreatment invertebrate taxa counts by lake								Grand total	Taxa diversity <sup>a</sup>
	CC	Crystal	Freds	G	Hope	Leisure Lake	Leisure Pond	Ranchero		
Annelida/Hirudinea	0	2	1	0	8	1	0	0	12	1
Amphipoda/Gammaridae	261	323	10	0	94	71	131	283	1,173	1
Annelida/Oligochaeta	7	7	3	18	6	1	1	1	44	1
Arachnida/Araneae	0	0	0	2	0	0	0	0	2	1
Annelida/Sangsue	0	0	0	0	0	0	0	0	0	0
Cladocera	967	50	4	210	7	642	309	144	2,333	1
Coleoptera/Chrysomelidae	1	0	0	0	0	0	0	0	1	1
Coleoptera/Dytiscidae	8	0	1	0	0	2	5	8	24	1
Coleoptera/Elmidae	0	0	0	0	0	0	0	1	1	1
Coleoptera/Gyrinidae	2	0	0	0	0	0	0	0	2	1
Coleoptera/Haliplidae	0	0	0	0	0	0	0	2	2	1
Coleoptera/Hydraenidae	0	0	0	0	0	0	1	0	1	1
Coleoptera/Hydrophilidae	0	0	0	0	0	0	2	2	4	1
Collembola/Isotomidae	0	1	0	0	0	0	0	0	1	1
Collembola/Sminthuridae	1	1	0	0	0	0	0	0	2	1
Copepoda	8	9	1	1	3	0	1	22	45	1
Crustacea/Ostracoda	20	3	2	1	2	2	31	34	95	1
Diptera/Ceratopogonidae	11	6	9	28	1	42	18	12	127	1
Diptera/Chironomidae	241	241	126	241	107	586	330	381	2,253	1
Diptera/Dixidae	5	0	0	0	0	0	0	2	7	1
Diptera/Empididae	1	0	0	0	0	0	0	0	1	1
Diptera/Nymphae	0	0	0	0	0	0	0	0	0	0
Diptera/Psychodidae	1	0	0	2	0	1	0	0	4	1
Diptera/Tabanidae	1	0	0	0	0	0	0	1	2	1
Diptera/Tipulidae	1	0	0	0	0	0	0	0	1	1
Ephemeroptera/Baetidae	0	2	0	0	0	0	0	0	2	1
Ephemeroptera/Caenidae	0	0	0	0	1	0	0	0	1	1
Ephemeroptera/Siphonuridae	0	0	0	0	0	0	0	0	0	0
Hemiptera/Aphidae	0	0	0	0	0	0	1	0	1	1
Hemiptera/Corixidae	0	6	0	0	1	6	7	1	21	1
Hydrachnidia/Hydracariens	19	2	48	40	0	15	26	34	184	1

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Order/Family	Posttreatment invertebrate taxa counts by lake								Grand total	Taxa diversity <sup>a</sup>
	CC	Crystal	Freds	G	Hope	Leisure Lake	Leisure Pond	Ranchero		
Hemiptera/Gerridae	0	0	0	0	0	1	0	0	1	1
Lepidoptera/Pyrilidae	0	0	0	0	0	3	0	0	3	1
Megaloptera/Sialidae	0	0	0	0	0	0	0	3	3	1
Mollusca/Lymnaeidae	0	0	0	0	0	0	2	0	2	1
Mollusca/Planorbidae	4	3	0	0	2	14	17	15	55	1
Mollusca/Sphaeriidae	50	95	8	5	101	49	16	71	395	1
Mollusca/Unionides	0	0	0	0	0	0	0	0	0	0
Nematoda/Nemathelminthes	0	17		7	5	3	2	2	36	1
Odonata/Aeshnidae	3	6	1	10	1	9	7	16	53	1
Odonata/Anisoptera	0	0	0	0	0	0	0	0	0	0
Odonata/Calopterygidae	10	2	1	22	1	36	12	6	90	1
Odonata/Coenagrionidae	0	3	0	0	0	0	0	0	3	1
Odonata/Cordulegastridae	0	0	0	9	0	0	0	0	9	1
Odonata/Corduliidae	5	0	0	6	0	9	6	10	36	1
Odonata/Lestidae	1	0	0	0	0	0	0	0	1	1
Odonata/Libellulidae	2	0	0	1	3	5	7	5	23	1
Plecoptera/Leuctridae	0	0	0	0	0	0	1	0	1	1
Trichoptera/Hydroptilidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Lepidostomatidae	0	1	0	0	0	0	0	1	2	1
Trichoptera/Leptoceridae	0	1	0	2	0	0	0	0	3	1
Trichoptera/limnephilidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Phylopotamidae	0	0	0	1	0	0	0	0	1	1
Trichoptera/Phryganeidae	0	1	0	0	0	0	0	0	1	1
Trichoptera/Polycentropodidae	0	0	0	0	0	0	0	0	0	0
Trichoptera/Psychomyiidae	0	0	0	2	0	1	0	0	3	1
Trichoptera/Rhyacophilidae	0	1	0	0	0	1	0	0	2	1
Trichoptera/Uenoidae	0	0	0	0	1	0	0	0	1	1
Diversity count (all lakes)									50	

*Note:* All data was provided courtesy by Dr. Alison Derry and undergraduates Nathan Juilliart and Anastasiya Zuko of the University of Quebec in Montreal, Department of Biological Sciences, CP 8888, 9 Succursale Centre-Ville, Montreal QC H3C 3P8 Canada. Each lake was surveyed at 8 sites.

<sup>a</sup> Taxa diversity definition: 1 = taxon is present; 0 = taxon is not present